5-2013

Vitamin D plus calcium supplementation among postmenopausal women : effect on risk of heart failure in the Women's Health Initiative.

Macarius Mwinisungee Donneyong
University of Louisville

VITAMIN D PLUS CALCIUM SUPPLEMENTATION AMONG POSTMENOPAUSAL WOMEN: EFFECT ON RISK OF HEART FAILURE IN THE WOMEN'S HEALTH INITIATIVE STUDY

by

MACARIUS MWINISUNGEE DONNEYONG

MPH

A Dissertation

Submitted to the faculty of the

School of Public Health and Information Sciences of the University of Louisville

In Partial Fulfillment of the Requirements

for the Degree of

DOCTOR OF PHILOSOPHY

Epidemiology

University of Louisville

Louisville, Kentucky

May 2013

VITAMIN D PLUS CALCIUM SUPPLEMENTATION AMONG POSTMENOPAUSAL WOMEN: EFFECT ON RISK OF HEART FAILURE THE WOMEN'S HEALTH INITIATIVE STUDY

by

MACARIUS MWINISUNGEE DONNEYONG

MPH

A Dissertation Approved on

April 16, 2013

By the following Dissertation Committee:

_____

Carlton A. Hornung, PhD, MPH
(Dissertation Director)

_____

JoAnn E. Manson, MD, DrPH

_____

Richard N. Baumgartner, PhD

_____

Kira C. Taylor, MS, PhD

_____

John A. Myers, MSPH, PhD

DEDICATION

I dedicate this dissertation to my parents, Mr. Moses N. Donneyong and Mrs. Florence A. Donneyong, who left no stone unturned in raising me into the man I am today. It is my hope that this work will inspire my daughter, Mwinmaalong and my siblings (Maurice, Mildred, Mabel, Madona, Medard, and Miriam), to achieve much greater things in their lives. Special thanks go to my wife, Matilda Tuuli Donneyong.

ACKNOWLEDGEMENTS

This dissertation would not have been completed successfully but for the hard work of my committee members who provided thorough criticisms and suggestions and demanded excellence from me. I will like to profusely thank Dr. Carlton A. Hornung, the chair of my dissertation committee, for looking beyond mere stereotypes to identify and nurture my intellectual potentials to the quality at which they are today. He never doubted my capabilities to jump over, rather than run under, the high bar of standards he has consistently set for students he mentored throughout his illustrious teaching career of about forty years. Dr. Hornung was more than an academic advisor, he was a father and a friend. I would not have been able to pursue this topic for a doctoral dissertation but for the invaluable input of Dr. JoAnn E. Manson who sponsored my access to the Women's Health Initiative (WHI) study data. Dr. Manson's in-depth knowledge of the WHI data and expertise in this field of study as well as her dedication were major catalyst for the quality of this dissertation. Please accept my humble gratitude, Dr. Manson. I am also indebted to Dr. Richard N. Baumgartner, my department chair and a committee member, for his keen eye to detail. He was also very supportive and encouraged me to pursue professional and academic opportunities beyond the school during my doctoral training. Many thanks also go to Dr. Kira C. Taylor who worked diligently to provide timely and detailed reviews. Her belief in my potentials and support motivated me to strive for excellence. Last but not least, I will like to deeply thank Dr. John A. Myers for his guidance, especially during the design and analysis stage of my dissertation.

Words cannot express how deeply I appreciate my beloved wife, Matilda Tuuli Donneyong, and precious daughter, Mwinmaalong Donneyong, for standing by me through thin and thick. Matilda's love, patience, understanding and encouragement were always in abundance throughout my doctoral studies. She made an immeasurable sacrifice to take care of our little girl all by herself just for me to earn this degree. Mwinmaalong's smiles and giggles were always enough to turn any gloomy day into a bright day and spurred me to complete my research in time. I would not have made it this far in life but for the unflinching love, support, encouragement, belief and prayers of my family back in Ghana. The wise words of my father, and personal hero, were ever present with me during sleepless nights to help me carry on. My mother's love, care and prayers assured me of the light that awaited at the end of the tunnel.

Lastly, I will like to thank all faculty and staff at the School of Public Health and Information Sciences (SPHIS) for their time, kindness and friendship. My time at SPHIS has been a memorable one because of people like Ms. Tammi Alvey Thomas, Dr. Muriel Harris, Dr. Richard Kerber, and Jason Banta.

ABSTRACT

VITAMIN D PLUS CALCIUM SUPPLEMENTATION AMONG POSTMENOPAUSAL WOMEN: EFFECT ON RISK OF HEART FAILURE IN THE WOMEN'S HEALTH INITIATIVE STUDY

Macarius Mwinisungee Donneyong

04/16/2013

This study evaluates the impact of vitamin D plus calcium supplementation as a primary intervention for heart failure (HF) prevention and examines whether preexisting conditions that are precursors of HF modify this relationship in a large cohort of postmenopausal women.

Analysis included 35,113 postmenopausal women (17,595 intervention, 17,518 control) aged 50 to 81 years enrolled in the randomized trial of vitamin D plus calcium (CaD) in the Women's Health Initiative (WHI) study. The women in this analysis cohort were free of HF at the time of randomization and during the first year of the trial. The intervention consisted of 1,000 mg/day of calcium and 400 IU/day of vitamin D3.

Incident HF cases over an average follow-up period of 7.13 (standard deviation, 1.33) years were identified from hospital discharge records and adjudicated by a physician committee. An intention-to-treat (ITT) approach was used to estimate hazard ratios (HR) and 95% confidence (CI) intervals from multivariable Cox Proportional Hazards regression models. A formal test of interaction between the intervention and a composite of risk factors that predispose towards the development of HF (hypertension, cardiovascular diseases, coronary heart diseases/events, and diabetes) and define 'Stage A' HF was performed.

CaD was associated with a non-significant 7% reduced risk of heart failure (HR = 0.92; 95% CI, 0.79 –1.06) in a multivariable model in the overall study cohort. However, CaD was associated with a clinically and statistically significantly lower risk (35%) of HF (HR = 0.65; 95% CI, 0.46 – 0.92; P = 0.01) among participants who were free of Stage A HF but neither a clinically nor a statistically significant effect among those with Stage A HF (HR = 1.02; 95% CI, 0.85 – 1.21; P = 0.87). Moreover, these effect estimates were not modified by baseline total (diet and supplements) vitamin D and calcium intake and persisted in a per-protocol and other sensitivity analyses.

These findings suggest that a low cost daily supplementation with vitamin D plus calcium may be an effective primary prevention strategy in postmenopausal without major cardiovascular precursors of HF but of little value in those with these risk factors.

TABLE OF CONTENTS

ACKNOWLEDGEMENTS iii

ABSTRACT iv

LIST OF FIGURES vii

LIST OF TABLES viii

Chapter ..... Page

TABLE OF CONTENTS viii

CHAPTER 1 4

BACKGROUND AND SIGNIFICANCE OF HEART FAILURE AND VITAMIN D DEFICIENCY AMONG ADULTS 4

1.1 Heart Failure 4

1.1.1 Definition and Classification of Heart Failure 4

1.1.2 Assessment of HF in Epidemiologic Studies 5

1.2 Epidemiology 9

1.2.1 General Population 9

1.2.2 Heart Failure among Women 12

1.2.3 Heart Failure with Preserved Ejection Fraction 12

1.3 VITAMIN D 13

1.3.1 Source and Synthesis of Vitamin D 14

1.3.2 Assessment of Vitamin D Status 16

1.3.3 Vitamin D Insufficiency: Issues on Definition and Prevalence 18

1.3.4 Causes and Prevention of Adult Vitamin D Deficiency 23

1.3.5 Prevention of Adult Vitamin D Deficiency 24
1.3.6 Consequences of Low Vitamin D Status on Non-musculoskeletal Conditions Among Adults 25
1.3.7 Vitamin D and Mortality 26
CHAPTER 2 31
EVIDENCE FOR ASSOCIATION BETWEEN VITAMIN D DEFICIENCY AND HEART FAILURE 31
2.1 Proposed Mechanisms by Which Vitamin D may Reduce CVD Risk 31
2.2 Association between Low Vitamin D Exposure and Heart Failure 34
2.3 Association between low vitamin D exposure and major HF risk factors 36
2.3.1 Cardiovascular Diseases (CVD) 38
2.3.2 Diabetes 41
2.3.3 Hypertension 43
CHAPTER 3 46
METHODOLOGY 46
3.1 Objectives 46
3.1.1 Main Objective 46
3.1.2 Specific Aims 47
3.2 Hypotheses 48
Primary Hypothesis 1 48
Primary Hypothesis 2 48
Secondary Hypothesis 49
3.3 Overview of the WHI study 50
Dietary Modification (DM) Trial 52
Hormone Therapy (HT) Trial 53
3.4 The Calcium plus Vitamin D (CaD) Trial 53
3.4.1 Eligibility Criteria 56
3.4.2 Characteristics of the CaD Trial Population 58
3.4.2 The CaD Trial Randomization 60
3.4.3 Adherence to Intervention 60
3.4.4 Data Collection Methods 62
3.5 Rationale for Analyzing the CaD Trial Data from the WHI Study 63

3.6 The WHI Study Data Access 64
3.7 Assessment of Heart Failure and Other Covariates 65
3.7.1 Ascertainment of Heart Failure Cases 65
3.7.2 Covariates and Effect Modifiers 65
3.7.3 Other Potential Covariates 69
3.8 Sample Selection and Power Analyses 70
3.8.1 Sample 70
3.8.2 Power Analyses 71
3.9 Statistical Analysis 76
3.9.1 Descriptive Statistical Analyses 77
3.9.2 Multivariable Analyses 77
3.10 Sensitivity Analyses 81
3.10.1 Per-protocol Analyses – 80% Adherence Rate 81
3.10.2 Estimating Hazard Ratios Independent of Noncompliance to Study Protocol by Inverse Probability of Censored Weights (IPCW) Method 82
3.10.3 Interaction between Intervention and Baseline Serum 25(OH)D Levels 83
CHAPTER 4 84
RESULTS 84
4.1 Characteristics of Participants during Randomization 84
4.1.1 Entire Study Population 84
4.1.2 Population stratified by baseline risk status for heart failure 87
4.2 Estimation of Risk of Heart Failure (Hazard Ratios) 92
4.2.1. The Cox Proportional Hazard Regression Model and Test of the Proportionality Assumption 92
4.2.2 Association between Calcium (1,000 Mg/Day) and Vitamin D (400 IU/Day) Supplementation and Incidence of Heart Failure 98
4.2.3 Association between Calcium (1,000 Mg/Day) and Vitamin D (400 IU/Day) Supplementation and Incidence of Heart Failure by Baseline Preexisting Diagnosed Cardiovascular Risk Factors of HF 101
4.2.4 Interaction between Intervention and Baseline Self-Reported Total (diet plus supplements) Vitamin D and Calcium Intake 108
4.3 Sensitivity Analyses 110
3.3.1 Per-protocol Analyses 110

4.3.2 Estimating Hazard Ratios Independent of Noncompliance to Study Protocol by Inverse Probability of Censored Weights (IPCW) Method 112
4.3.4 Interaction between Intervention and Baseline Serum 25(OH)D Levels 113
CHAPTER 5 114
DISCUSSION 114
5.1 Summary of Results 114
5.1.1 Effect of Intervention in the Overall CaD Cohort 116
5.1.2 Effect of the Intervention in Populations with Preexisting Baseline Cardiovascular Risk Factors of Heart Failure 119
5.1.3 Effect of the Intervention in Populations without Preexisting Baseline Cardiovascular Risk Factors of Heart Failure 123
5.2 Limitations 126
5.3 Strengths 127
5.4 Public Health Implications 128
CONCLUSION 130
RECOMMENDATIONS 132
REFERENCES 133
APPENDIX 151
WHI form for ascertaining heart failure – Form 121, page 4 of 7 155
Table 2 156
Interaction between intervention and use of cardiovascular medication 156
CURRICULLUM VITAE 157

LIST OF FIGURES

FIGURE PAGE

FIGURE 1. SCHEMATIC REPRESENTATION OF VITAMIN D SYNTHESIS IN HUMANS.....16

FIGURE 2. POTENTIAL MECHANISMS FOR CARDIOVASCULAR EFFECTS OF VITAMIN D DEFICIENCY.....32

FIGURE 3. OVERLAP OF PARTICIPANTS ENROLLED IN THE THREE CLINICAL TRIALS (HT, DM, AND CAD) OF THE WOMEN'S HEALTH INITIATIVE (WHI) STUDY. 52

FIGURE 4. PARTICIPANT RECRUITMENT AND SELECTION FOR THE CAD TRIAL AND ANALYSIS 55

FIGURE 5. ADHERENCE RATE TO STUDY PROTOCOL IN THE VITAMIN D PLUS CALCIUM TRIAL OF THE WHI.....61

FIGURE 6. PLOT OF STATISTICAL POWER WITH DETECTABLE HAZARD RATIOS FOR THE ENTIRE CAD TRIAL POPULATION (N=35,113) ANALYZED.....74

FIGURE 7. PLOT OF STATISTICAL POWER WITH DETECTABLE HAZARD RATIOS FOR THE CAD TRIAL POPULATION AT LOW RISK FOR HEART FAILURE DURING RANDOMIZATION (N= 18,097).....75

FIGURE 8. PLOT OF STATISTICAL POWER WITH DETECTABLE HAZARD RATIOS FOR THE CAD TRIAL POPULATION AT HIGH RISK FOR HEART FAILURE DURING RANDOMIZATION (N= 17,016) ANALYZED.....76

FIGURE 9. KM CURVES COMPARING THE CUMULATIVE INCIDENCE OF HF BETWEEN THE INTERVENTION AND PLACEBO ARMS DURING FOLLOW-UP PERIOD FOR THE OVERALL CAD COHORT. 93

FIGURE 10. SCHOENFIELD RESIDUAL CURVE TO EVALUATE THE PROPORTIONAL HAZARDS ASSUMPTION BETWEEN THE INTERVENTION AND PLACEBO IN THE OVERALL CAD COHORT. 94

FIGURE 11. KM CURVES COMPARING THE CUMULATIVE INCIDENCE OF HF BETWEEN THE INTERVENTION AND PLACEBO ARMS DURING FOLLOW-UP PERIOD FOR THE GROUP WITHOUT PREEXISTING DIAGNOSED CARDIOVASCULAR RISK (CV) FACTORS OF HF....95

FIGURE 12. SCHOENFIELD RESIDUAL CURVE TO EVALUATE THE PROPORTIONAL HAZARDS ASSUMPTION BETWEEN THE INTERVENTION AND PLACEBO IN THE LOW RISK POPULATION.....96

FIGURE 13. KM CURVES COMPARING THE CUMULATIVE INCIDENCE OF HF BETWEEN THE INTERVENTION AND PLACEBO ARMS DURING FOLLOW-UP PERIOD FOR THE GROUP WITH PREEXISTING DIAGNOSED CARDIOVASCULAR RISK (CV) FACTORS OF HF.....97

FIGURE 14. SCHOENFIELD RESIDUAL CURVE TO EVALUATE THE PROPORTIONAL HAZARDS ASSUMPTION BETWEEN THE INTERVENTION AND PLACEBO IN THE HIGH RISK POPULATION.....98

FIGURE 15. KM CURVES COMPARING THE CUMULATIVE INCIDENCE OF HEART FAILURE BETWEEN POPULATIONS STRATIFIED BY THE PRESENCE OR ABSENCE OF BASELINE CARDIOVASCULAR RISK FACTORS OF HEART FAILURE. 105

FIGURE 16. FOREST PLOT OF HAZARD RATIOS FOR EFFECT OF VITAMIN D PLUS CALCIUM SUPPLEMENTATION ON HEART FAILURE. 108

FIGURE 17. PLOT OF NUMBER NEEDED TO TREAT BY VITAMIN D AND CALCIUM SUPPLEMENTATION IN POPULATIONS WITHOUT PREEXISTING CARDIOVASCULAR RISK FACTORS OF HEART FAILURE. 129

LIST OF TABLES

TABLE.....PAGE

1. WHI INCLUSION AND EXCLUSION CRITERIA.....57

2. SUMMARY OF DEMOGRAPHIC CHARACTERISTICS OF PARTICIPANTS RECRUITED INTO THE CAD TRIAL BEFORE RANDOMIZATION.....59

3. FREQUENCY OF MISSING VALUES FOR SELECT COVARIATES BY STUDY ALLOCATION.....71

4. COMPUTED CRITICAL HAZARD RATIOS FOR THE THREE DIFFERENT POPULATIONS FROM PS (VERSION 3.0.43).....73

5. STUDY PROTOCOL ADHERENCE RATE.....82

6. DISTRIBUTION OF PARTICIPANTS' CHARACTERISTICS BY TREATMENT GROUP AT BASELINE - THE VITAMIN D AND CALCIUM (CAD) TRIAL OF THE WOMEN'S HEALTH INITIATIVE (WHI) STUDY, 1995 – 2005.....85

7.BASELINE CHARACTERISTICS OF PARTICIPANTS WITHOUT PREEXISTING CARDIOVASCULAR RISK FACTORS OF HEART FAILURE (N = 18,097) BY TREATMENT GROUP - THE VITAMIN D AND CALCIUM (CAD) TRIAL OF THHE WOMEN'S HEALTH INITIATIVE (WHI) STUDY, 1995 – 2005.....88

8. BASELINE CHARACTERISTICS OF PARTICIPANTS WITH PREEXISTING CARDIOVASCULAR RISK FACTORS OF HEART FAILURE (N = 17,016) BY TREATMENT GROUP - THE VITAMIN D AND CALCIUM (CAD) TRIAL OF THE WOMEN'S HEALTH INITIATIVE (WHI) STUDY, 1995 – 2005.....90

9. ASSOCIATION BETWEEN CALCIUM (1000 MG/DAY) AND VITAMIN D (400 IU/DAY) SUPPLEMENTATION AND INCIDENCE OF HEART FAILURE, WHI STUDY, 1995 – 2005 .100

10. OVERLAP OF BASELINE CARDIOVASCULAR RISK FACTORS OF HEART FAILURE 103

11. DISTRIBUTION OF PARTICIPANTS BY MINIMUM NUMBER OF CARDIOVASCULAR (CV) RISK FACTORS OF HEART FAILURE.....104

12. ASSOCIATION BETWEEN CALCIUM (1000 MG/DAY) AND VITAMIN D (400 IU/DAY) SUPPLEMENTATION AND HEART FAILURE STRATIFIED BY BASELINE PREEXISTING

DIAGNOSED CARDIOVASCULAR RISK FACTORS OF HEART FAILURE, WHI STUDY, 1995 – 2005 107

13. ASSOCIATION BETWEEN CALCIUM (1000 MG/DAY) PLUS VITAMIN D (400 IU/DAY) SUPPLEMENTATION AND HEART FAILURE STRATIFIED BY BASELINE VITAMIN D AND CALCIUM INTAKES, WHI STUDY, 1995 – 2005 110

14. ASSOCIATION BETWEEN CALCIUM (1000 MG/DAY) AND VITAMIN D (400 IU/DAY) SUPPLEMENTATION AND HEART FAILURE AMONG THE STUDY POPULATION WITH AT LEAST 80% ADHERENCE TO STUDY MEDICATION, WHI STUDY, 1995 – 2005..... 112

15. ESTIMATED HAZARD RATIOS FOR THE ASSOCIATION BETWEEN THE INTERVENTION AND RISK OF HEART FAILURE INDEPENDENT OF COMPETING RISKS AND NONCOMPLIANCE TO STUDY MEDICATION THROUGH THE INVERSE PROBABILITY OF CENSORED WEIGHTS METHOD (IPCW) 113

16. DISTRIBUTION OF SELECTED BASELINE COVARIATES BY THE PRESENCE AND ABSENCE OF BASELINE CARDIOVASCULAR RISK FACTORS OF HEART FAILURE 121

INTRODUCTION

Heart failure (HF) is a major public health problem with enormous health care cost, mortality, and functional limitation (1). Currently, about 5.8 million adult Americans live with HF, and half of this population will die within five years of diagnosis (2, 3). The prevalence and mortality rate of HF are likely to continue because HF carries a lifetime risk of 20% after 40 years of age (4). Given the high associated healthcare cost and burden on HF survivors, it is a high priority to identify interventions for HF prevention. Some have suggested that dietary supplementation with vitamin D may be a potential inexpensive primary prevention measure that can be explored to mitigate this epidemic.

The role of vitamin D in HF pathogenesis in humans is not well established. Indeed there are few published studies designed with the ability to test a causal hypothesis. However, several observational studies have examined associations between cardiometabolic outcomes, including HF, and vitamin D deficiency/insufficiency. While several of these studies suggest an association between low 25(OH) vitamin D levels and increased risk of cardiometabolic outcomes, some inconsistencies still exist (5-9). Animal models, however, provide compelling evidence to demonstrate a plausible pathophysiological role of vitamin D in the etiology of HF (10-12). Hence, one of the existing knowledge gaps on the role of vitamin D in HF is whether a protective effect can

be demonstrated in humans. To bridge this gap, it is imperative to analyze data from a randomized controlled trial (RCT) of vitamin D among a primary prevention cohort free of HF at baseline. Data from the Women's Health Initiative (WHI) clinical trial of combined vitamin D and calcium is an ideal resource for this type of analysis.

While results from RCTs are inconsistent for the associations between some cardiovascular diseases (CVDs) and vitamin D alone, or combined with calcium, data from observational longitudinal studies tend to be more consistent on reporting inverse associations (5, 6, 8, 9). To our knowledge, however, none of these RCTs included CVD as a primary pre-specified outcome. Among HF patients, supplementation with vitamin D has been observed to have modest effects on some prognostic outcomes such as improved ventricular function and N-terminal fragment brain natriuretic peptide (NT-proBNP) levels (8). Data on vitamin D supplementation among chronic kidney disease patients provides vital information about the plausible pathophysiological mechanism by which low vitamin D may initiate cardiac malfunctioning (13-17). The evidence from these studies, albeit modest, has stimulated intense interest in assessing the possible benefits of vitamin D in HF pathogenesis.

This dissertation investigates the effect of combined supplementation with vitamin D and calcium as a primary HF prevention among postmenopausal women. It also examines whether the composite of preexisting conditions (e.g., hypertension, cardiovascular diseases, coronary heart diseases/events, and diabetes) that are precursors of HF modify the effect of vitamin D plus calcium supplementation on HF incidence. We hypothesize that vitamin D plus calcium supplementation will reduce HF incidence in the

overall population; this risk reduction will be stronger in the group of women with these preexisting HF precursors than in those free of these precursors. The American College of Cardiology (ACC) classifies individuals with any of these risk factors (hypertension, cardiovascular diseases, coronary heart diseases/events, and diabetes) who have not yet developed structural abnormalities like left ventricular dysfunction or hypertrophy or geometric chamber distortion as 'Stage A' heart failure and those who demonstrate any of these structural abnormalities as 'Stage B'. While Stages A and B heart failure are not HF per se, these designations enable healthcare providers to identify individuals at the greatest risk of HF for early interventions (18). In the general female population between the ages of 40 – 89 years, hypertension, diabetes, and CVDs are associated with population attributable risks (for HF) of 59%, 12%, and 26%, respectively (19). Interestingly, these precursors of HF have also been found to be inversely correlated with serum vitamin D levels (5-9).

In line with the American Heart Association (AHA)/American College of Cardiology (ACC) recommendation for targeting HF interventions in those with these risk factors and classified as stage A or B, it is work investigating whether supplementation with vitamin D plus calcium could be an effective intervention. Such an inexpensive intervention, if effective, could be a novel public health strategy to mitigate the unabated rise of healthcare costs, mortality, functional limitation, and impairment of quality of life associated with HF.

CHAPTER 1

BACKGROUND AND SIGNIFICANCE OF HEART FAILURE AND VITAMIN D DEFICIENCY AMONG ADULTS

1.1 Heart Failure

1.1.1 Definition and Classification of Heart Failure

There is no one specific definition of HF owing to lack of consensus on diagnostic criteria. Several definitions have been proposed over the past decades. However, the American Heart Association and American College of Cardiology (AHA/ACC) defined heart failure (ICD-10: 150) as "a complex clinical syndrome that can result from any structural or functional cardiac disorder that impairs the ability of the ventricle to fill with or eject blood" (20). This definition is more comprehensive than previous ones which were limited to either the symptoms alone or physical signs of fluid retention alone (21-23).

Fishberg first reported – over 70 years ago – the existence of two forms of heart failure (24). These were later termed diastolic and systolic heart failure. While the definition of diastolic heart failure (DHF) has been controversial, Zile and Brutsaert (2002) recently defined DHF as a condition in which the "ventricular chamber is unable

to accept an adequate volume of blood during diastole at normal diastolic pressures and at volumes sufficient to maintain an appropriate stroke volume" (25). In contrast, systolic heart failure (SHF) was defined by Braunwald (1980) as "'a pathophysiological state in which an abnormality of cardiac function is responsible for the failure of the heart to pump blood at a rate commensurate with the requirements of the metabolizing tissues" (26). Both definitions, however, only describe structural and functional abnormalities (27).

HF has subsequently been defined by the left ventricular ejection fraction (LVEF) which is the ratio of end systolic volume (blood ejected during systole) to end-diastolic volume (blood in the ventricle at the end of diastole). Heart failure with preserved left ventricular ejection fraction (HPLVEF) and heart failure without preserved left ventricular ejection fraction are the current terminologies. HPLVEF occurs when a higher filling pressure is required to achieve normal end-diastolic volume in the left ventricle even though the "normal" pumping function of the left ventricle is maintained (28, 29, 30). What constitutes a preserved LVEF is a contentious issue in the scientific community. While there is no consensus on the threshold for LVEF to distinguish the two forms of HF, LVEF greater than 40% to 50% is widely referred to as preserved (21, 31, 32). Distinguishing HF based on LVEF has important epidemiological and great clinical importance as discussed in subsequent sections.

1.1.2 Assessment of HF in Epidemiologic Studies

This section reviews existing HF assessment methods in epidemiologic studies. The most commonly used clinical diagnostic criteria are those used in the Framingham

Heart Study (FHS), the European Society of Cardiology (ESC), and the Boston clinical diagnostic criteria of heart failure (21, 33, 34). The FHS establishes definite heart failure diagnoses based on two major or one major and two minor criteria assessed by physical examination, clinical tests, and chest X-ray (34). The ESC criteria involve tests based on electrocardiogram, chest X-ray, and laboratory results on total blood count, serum electrolytes and creatinine, estimated glomerular filtration rate, glucose, liver function, and urinalysis (21). The Boston criteria classifies patients as possible, definite, or unlikely heart failure based on history of dyspnea, physical examination of heart rate, jugular-venous pressure, and lung function, and a chest X-ray (33). Detailed description of each of these criteria can be found in the appendix.

Heart failure assessment in primary data collection

There is a lack of consensus on the HF diagnostic criteria with the least misclassification bias – the criterion with the highest diagnostic validity (35). While this is a matter of concern, few studies have evaluated and compared, head-to-head, which of the existing criteria should be the most preferable for diagnosing HF in the community.

Bari et. al. (2004) conducted a comparative validation study of established criteria for HF ascertainment among 553 elderly community dwellers of mean age 73 ± 0.3years (36). The FHS, Boston, and ESC criteria were compared on the prevalence and diagnostic concordance, construct validity, and predictive validity of heart failure incidence. The agreement between these criteria ranged from very poor ($k = 0.05$) to moderate ($k = 0.59$) even though the prevalences based on these criteria were similar for Framingham (11.9%), Boston (10.7%), and ESC (9.0%); the prevalence was much higher (20.8%)

when assessed by the Gothenberg criteria. However, the Boston and Framingham criteria were superior over Gothenberg and ESC when discriminating between HF and non-HF in terms of the construct validity. Components of the construct validity included abnormalities in cardiac structure and function (left ventricular mass index, left ventricular ejection fraction, and left atrial systolic dimension), and global functional status (lower extremity mobility disability, 6-minute walk test, and summary performance score). In terms of predictive validity the Boston criteria were superior over the rest; components of the predictive validity were cardiovascular mortality, incident basic activities of daily living disability, and HF-related hospitalizations (36). This study, Bari et. al. (2004), highlights the inconsistency in epidemiologic results from population-based studies. Even though the study suggests the Boston criteria has the best construct validity most accurately predicts cardiovascular death, disability, and hospitalization, this is by no means a final verdict on which method should the most preferable. The relatively small sample (N = 553) and old population (mean age, 73.0 ± 0.3 years) limits the application of this study.

In the other study, Schellenbaum et. al. (2004) compared the incidence and survival of HF among a cohort of 875 hospitalized HF patients – sampled from the Cardiovascular Heart Study (CHS) population - after a HF onset as defined by the FHS or CHS central adjudication criteria, or both (37). While there were no observed differences for mortality (HRs 0.87 vs 0.89) between these criteria, the FHS criteria identified as twice as many incident HF cases (n = 140) as the CHS criteria (n = 71). This suggests the CHS centrally adjudicated process might be more conservative than the FHS; the FHS criterion was not complemented with central adjudication process by a panel of

physicians. Likely, there would have been relatively higher misclassification of incident cases identified by the FHS approach. The shortfalls of this study were based on the use of death as a HF end-point since little differences could be detected in this elderly (mean age of 80 yrs) sample. The comparison was also weakened by the significantly larger proportion of missing data reported for the FHS criteria. Notwithstanding these shortcomings, the study highlights the importance of central adjudication of HF cases for epidemiologic studies. In summary, there is no current "gold standard" for identifying incident HF cases with minimum misclassification bias, central adjudication by a committee is essential for assessing HF in epidemiological studies (37-40).

Heart failure assessment in secondary data collection - hospital discharge diagnosis

Epidemiological studies often utilize hospital discharge data for estimating the incidence and secular trends of heart failure because of their relative ease of accessibility and lower cost to the investigator. However, hospital discharge data are influenced by reimbursement codes as well as inconsistencies in coding practices (41). Heckbert et. al. (2004) reported moderate agreement (k = 0.56, 095% CI:0.53 – 0.59) between HF cases identified by hospital discharged codes and by a local adjudication committee of the WHI study based on data from 34,016 participants with reported cardiovascular endpoints (42). In another study, Schellenbaum et. al. (2006) compared HF incidence rates between hospital discharge diagnosis (ICD-9 codes: 428, 997.1, 425, 402.01, 402.11, 402.91, and 398.91.) and adjudication based on data from the Cardiovascular Health Study and found that the incidence of HF was higher with the hospital discharge criterion compared to the central adjudication criterion, 24.6 vs 17.1 per person-years (38). In summary, there is no current "gold standard" for identifying incident HF cases with minimum misclassification

bias, central adjudication by a committee is essential for population studies because this method has a relatively higher specificity (37-40, 42).

1.2 Epidemiology

HF continues to be a major public health concern even though the incidence of heart failure (HF) appears to have plateaued over the last decade owing to decline of major determinants such as ischemic heart disease and control of hypertension (43). With a current estimated prevalence of 2 – 3% in the US, this pool of 5.8 million HF patients is likely to grow as a result of rapidly aging population and improved management that prolongs survival (43). Worldwide, it is estimated that 23 million individuals are affected with HF (44). Consequently, HF will continue to exact a large toll on public health resources and pose a major health burden on individuals and their families.

The current prevalence and incidence estimates might be an under-estimation of the real impact of HF because of lack of consensus on the HF assessment criteria at the population level. Additionally, reported prevalence and incidence rates among females and the elderly are not consistent with the national figures. Under-reporting and poor diagnoses are also likely sources of under-estimation of HF at worldwide level.

1.2.1 General Population

Prevalence

The American Heart Association's 2011 update on Heart diseases and Stroke indicated about 5.8 million (2.42%) adult Americans reported they have been diagnosed with HF based on data from NHANES (2). The age-adjusted self-reported prevalence of a

relatively younger (≥18 years) population of the National Health Interview Survey was 1.2% in 1999 (45). In the Olmsted County (MN) study, 2.2% of the population (n = 2,024) aged 45 years and above were assessed to be living with HF based on the FHS criteria (46). However in an older population of Medicare beneficiaries aged 65 years and above, the prevalence of HF in 2003 was 12.1%; diagnostic records indicated the prevalence was higher in men (12.9%) than women (11.5%) (47). In the Rotterdam study, the prevalence, as assessed by the ESC method, was 7.0% among a population with an average age of 74.5 years. In these and other studies, the prevalence increased with older age and with male gender.

Incidence

The incidence of HF is reported to range from 2 to 5 per 1000 person-years depending on the method of HF case ascertainment and population. After an average follow-up of 5.5 years of an elderly population (mean age 73.5 years) in the CHS, the incidence of HF was 19.3/1,000 person-years. Again, as for prevalence, the incidence rate rose with increasing age and male sex (48). In the FHS, the incidence was slightly higher in men (5.64/1000 person-years) which remained unchanged between 1950 – 1999 compared to females (3.27/1000 person-years) who experienced a decline of about 31 – 40% during the same period (49). In the Olmsted County, MN study, there was no temporal decline in HF incidence. Again, the incidence was relatively higher in males compared to females, 3.78 vs 2.89 per 1000 person-years (3). There is insufficient evidence to suggest there is or might soon be a decline in HF incidence in spite of these data suggesting a plateauing of incidence rates.

Mortality

The five-year HF mortality is still high even though there has been a decline in the last three devades (2, 3, 49). HF tends to have poor survival prognosis after diagnosis – mortality increases with post-diagnostic time. In 2007, 11% of deaths had HF mentioned on the certificate, of which every 1 out of 5 deaths was due to HF. About 60% of these deaths occurred in females (2). These national data are based on mortality due to HF and do not clarify survival time after onset of HF (49). Reviews by Hsich et. al (2009) and Shah et. al. (2009) suggested females have better five-year survival compared to males (50, 51). However, these sex differences tend to narrow when investigated in older age populations and there is no difference in the overall survival trends.

Data from the FHS showed 30-day, one-year, and five-year mortality rates as 11.5%, 26%, and 52% respectively for the period between 1990 and 1999. The 5-year mortality rate declined by 16% and 21% from 1950 – 1990 for both males and females respectively (49). This has been confirmed by data from the Olmsted County, MN study where an overall decline of 16% in HF 5-year mortality was observed between 1979 and 2000. In this population however, there were age and sex differences in the decline of mortality. Improvement in survival was highest among younger ages (< 70 years) for both sexes but remained higher among males. In contrast, improvement in survival for older males was less, compared to younger males while older females did not improve in survival over time (3). These results have been corroborated in a study by Barker et. al. (2006) among a well defined older population (≥ 65 years) based on administrative data (52). This retrospective cohort study suggested while HF incidence increased between

1970s and 1990s, the overall five-year survival for the same period improved, with males experiencing higher incidence and improved survival compared to females (52).

The five-year age-adjusted mortality due to HF declined over the past 4 – 5 decades with little or no differences between the sexes. This trend was consistent in all the data reviewed in spite of population structure differences between these data. This contrasts with the higher HF incidence figures associated with males, as presented in the previous section. With equity in the survival rates between the sexes, it suggests more males than females are surviving with HF owing to some biological and therapeutic differences.

1.2.2 Heart Failure among Women

While the studies reviewed suggest the incidence of HF among males may be higher than among females, they failed to distinguish between systolic HF and heart failure with preserved left ventricular ejection fraction (HPLVEF). More females than male HF patients tend to have HPLVEF. The observed HPLVEF prevalence difference between males and females is crucial for interpreting epidemiologic data of HF in the general population.

1.2.3 Heart Failure with Preserved Ejection Fraction

Owan and Redfield (2005) reported the prevalence of HPLVEF among overt HF patients aged 25 years and above to range between 40 – 71% (53). The prevalence of HPLVEF increased over a fifteen-year period among 6,076 patients discharged with HF. In this study, by Owan et. al. (2006), HF was validated using the FHS and clinical

criterion (attending physician's recorded diagnosis) with HPLVEF defined as HF with LVEF ≥ 50% (54).

Data on mortality differences between HF with reduced ejection fraction and HPLVEF have been inconsistent. In their review of the current epidemiologic data on mortality associated with HF diagnosis with reduced and preserved ejection fractions, Owan and Redfield (2005) could not find sufficient evidence supporting better survival for either form of HF (53). However, among the CHS cohort, the population attributable risk of mortality was higher for those with impaired left ventricular function, EF <45% (7.5%) compared to those with normal ventricular function, EF >=55% (5.9%) and may partly explain why females have higher HF mortality (48). It should be observed that the epidemiologic studies reviewed in this publication used all-cause mortality as their outcome instead of CVD mortality or, more precisely, HF-specific mortality, which would have been more informative on the pathophysiological differences between the two forms of HF. However, Owan et. al. (2006) reported HPLVEF (EF ≥ 50%) to be associated with slightly lower risk of mortality (HR = 0.96; 95%CI: 0.92 – 0.99) compared to those with reduced ejection fraction (EF ≤ 50%). In contrast, when secular trends in survival were investigated, HF with reduced ejection fraction was associated with improved survival compared to HPLVEF (54). This paradoxical finding may be attributable to the lack of existing proven effective therapies for HPLVEF (35, 54).

1.3 VITAMIN D

Vitamin D is a curious vitamin; it mimics the action of a hormone in humans and is the only vitamin that is synthesized in the body with exposure to solar radiation.

Historically, vitamin D was discovered as a result of high prevalence of rickets in children during the industrial revolution in northern Europe. This condition was recognized to be a result of reduced sun exposure owing to high air pollution. These events spurred the search for a cure of rickets and the eventual discovery of vitamin D (55).

1.3.1 Source and Synthesis of Vitamin D

When it was discovered humans could synthesize vitamin D from the sun's radiation, efforts were made to artificially produce vitamin D. Ultra-violet (UV) lamps were used for treating rickets both in humans and animals. Subsequently, foods, especially milk, were fortified with vitamin D through irradiation. Currently, the fortification of foods with vitamin D has been expanded to include fruit juices, cereals, and dairy products. However, there are only a few naturally occurring sources of vitamin D. Sea foods, especially oily fish like wild salmon have abundant reserves of vitamin D. And now, vitamin D supplements are readily available. Vitamin D_2 and D_2 are the commercially available vitamin D supplements. Vitamin D_2, also known as ergocalciferol, is derived from yeast/fungus while D_3, also known as colecalciferol is synthesized from animal sources through a process similar to how humans synthesize vitamin D from sunlight (56).

Humans and other land vertebrates have abundant concentrations of 7-dehydrocholesterol, a precursor of cholesterol, in their skin. Figure 1 illustrates a schematic synthesis of vitamin D from 7-dehydrocholesterol in the skin through exposure to UV radiation as well as from diet/supplements (57). When the skin surface is exposed

to UV-B from the sun, 7-dehydrocholesterol undergoes chemical transformation to become pre-vitamin D_3. Pre-vitamin D_3 is highly unstable and undergoes further transformation (rearrangements of it's double bonds to be precise) to produce vitamin D_3. Vitamin D_3 then binds to a vitamin D binding protein in the epidermal bed and is transported to the liver for metabolism. Vitamin D_2 and D_3 can also be acquired from diet and nutritional supplements. All these forms of exogenous vitamin D are metabolized in the liver to form 25(OH)D, an intermediate metabolite of vitamin D which is used as a vitamin D biomarker. When transported to the kidneys, the enzyme 25-hydroxyvitamin D-1α-hydroxylase converts 25(OH)D to its active metabolic form, 1,25-dihydroxyvitamin D ($1,25[OH]_2D$) (58-60).

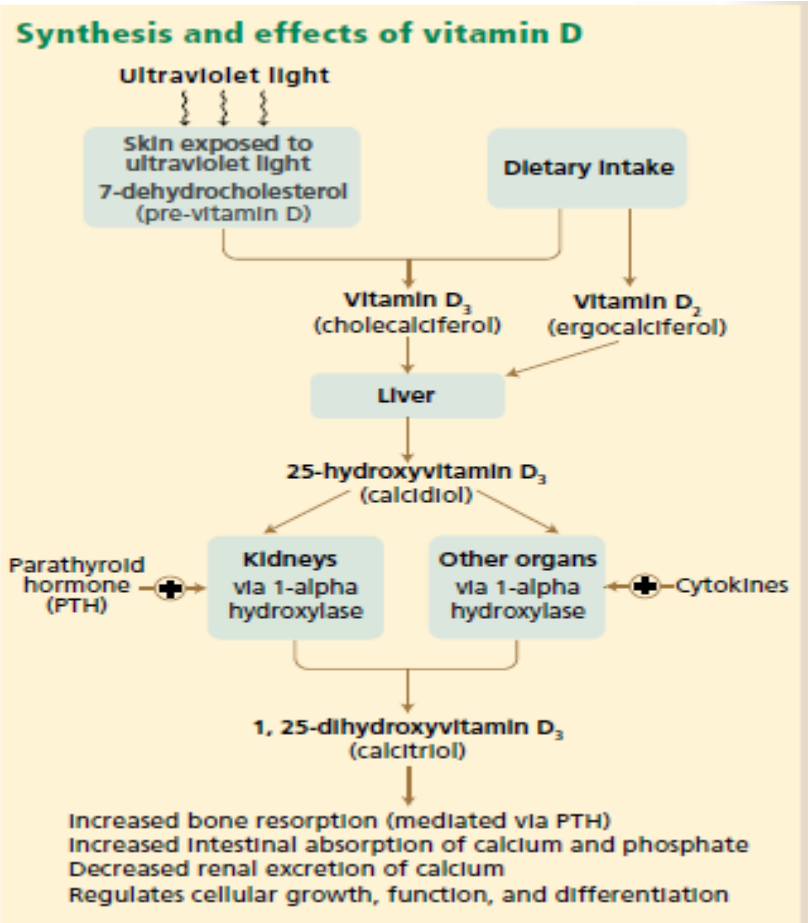


Figure 1. Schematic representation of vitamin D synthesis in humans.

Reference: Hajjar V, Depta JP, Mountis MM. Q: Does vitamin D deficiency play a role in the pathogenesis of chronic heart failure? Do supplements improve survival? Cleve Clin J Med 2010;77(5):290-3.

1.3.2 Assessment of Vitamin D Status

Vitamin D status in populations is based on serum concentrations of the vitamin D biomarker, 25(OH)D. In their guidelines for vitamin D deficiency, Holick et. al. (2011) recommended against screening for vitamin D deficiency based on the active metabolic form, $1,25(OH)_2D$, because its concentrations are regulated by plasma parathyroid

hormones (PTH) and intestinal calcium and phosphorus levels (58-61). Instead, 25(OH)D levels are preferred for screening populations for vitamin D deficiency. Alternatively, vitamin D status in populations can be measured by exogenous vitamin D intake. This method, based on data from self-reported dietary and supplement intake, is less precise than that based on measured serum levels of endogenous 25(OH)D levels. Serum 25(OH)D levels reflect the amount of vitamin D ingested from both diet and supplements, as well as that synthesized endogenously. However, in populations where the sun is the major source of vitamin D, vitamin D status assessment based on serum 25(OH)D levels is not an accurate method.

Firstly, serum 25(OH)D concentrations are greatly influenced by seasonal variation in sun exposure. As expected, individual and population-based 25(OH)D levels reach their peak and nadir in the summer and winter months respectively (62). This makes baseline or one time measurements of 25(OH)D inaccurate for estimating the vitamin D status of subjects (63). This shortfall is corrected in population based studies by statistically controlling for season of blood draw (63). Additionally, validation of serum 25(OH)D levels can be conducted by taking serial measurements that span all seasons.

Second, 25(OH)D concentrations are highly variable because 25(OH)D has a relatively short circulation half-life (about 3 weeks) (64, 65). This may reduce its predictive value for estimating the relationship between vitamin D status and diseases. As suggested above, taking serial measurements of subjects in longitudinal studies is one

way to overcome this flaw. However, it will time consuming and expensive to routinely screen patients for their vitamin D status in large population-based studies.

Serum 25(OH)D measurement is still the preferred method for estimating vitamin D status in epidemiological studies in spite of the drawbacks mentioned above. Both vitamin D_3 (cholecalciferol) and D_2 (ergocalciferol) and the active metabolite of vitamin D, $1,25(OH)_2D$, have shorter circulation half-lives compared to 25(OH)D (66, 67) making them more difficult to assay. Moreover, they don't reflect total vitamin D from both endogenous and exogenous sources. Total vitamin D intake has worse reliability compared to 25(OH)D and the other biomarkers because this method is subject to recall biases of all foods and the quantity eaten in the past. Also, the amount of vitamin D endogenously synthesized is missed, which leads to underestimation of vitamin D status because sun exposure is still a major source of vitamin D for both young and old community dwelling adults (68). In addition, the metabolism of total vitamin D intake from diet and supplements is affected by race, age, sun exposure, obesity, physical activity, and region of residence (69, 70).

Hence in assessing vitamin D status of populations in epidemiologic studies, it is advisable to incorporate both total vitamin D intake and amount of sun exposure in the region of residence together with the preferred biomarker of vitamin D status, 25(OH)D (63).

1.3.3 Vitamin D Insufficiency: Issues on Definition and Prevalence

The definition of adult vitamin D deficiency has been debated over the years in the scientific community. This debate has been partly fueled by the emergence of non-

musculoskeletal functions of vitamin D. However, a threshold of serum vitamin D (25[OH]D) levels less than 20 ng/mL (50 nmol/L) is widely accepted as indication of adult vitamin D insufficiency (71). Although some have defined vitamin D insufficiency 25(OH)D levels as <30 ng/mL, this definition has been challenged by the IOM (60, 61, 72-74). Definitions used by different groups have been based on evidence from studies that reported absorption of intestinal calcium is optimized at 25(OH)D levels above 32 ng/mL in postmenopausal women and by evidence that adult parathyroid hormone (PTH) concentrations reach their lowest point at 25(OH)D levels between 30 ng/mL and 40 ng/mL (75-78). While these vitamin D concentration thresholds are widely employed for screening and surveillance purposes, investigators often define their own cut-off values in studying associations between vitamin D status and health outcomes.

Based on data from NHANES (2001-2004), 6% of the US population aged 12 years and older had severe low vitamin D status, 25(OH)D < 10ng/mL (79). Approximately three-quarters of the population, 71 % (95%CI: 68-73), had 25(OH)D concentrations between 10 ng/mL and 30 ng/mL. On average, non-Hispanic whites had relatively higher mean 25(OH)D levels (≈ 26 ng/mL) compared to non-Hispanic blacks, ≈ 15 ng/mL. On the other hand, there were no observed differences in the mean 25(OH)D levels (≈ 24 ng/mL) between the sexes. While there were no age-related differences in mean 25(OH)D levels, age was inversely related to 25(OH)D concentrations among non-Hispanic whites but not in non-Hispanic blacks.

Recent analysis of the 2005 – 2006 NHANES data restricted to participants aged 20 years and above (N=4,495) reported 41.6% of this population had vitamin D

insufficiency (25[OH]D < 20 ng/mL) with even higher rates among Blacks (82.1%) and Hispanics (62.9%). While there were no observed significant differences in 25(OH)D between different age groups, the prevalence of vitamin D insufficiency was highest among 55 – 59 years (49%) and 60 – 65 years (47%) (80). While both analyses of two separate NHANES datasets concluded the prevalence of vitamin D deficiency is high in the US, the prevalence is likely to be much higher because populations living in the northern latitudes of the US were not sampled during winter months.

It is not very clear why these disparities in 25(OH)D occurred, but Ginde et. al. (2009) posited the difference could be as a result of varying time spent indoors. Their analysis of the NHANES data however did not explore differences in duration of outdoor activities between the sexes and age categories. The explanation given by Ginde et. al. starkly contrasts with the results of Scragg and Camargo (2008) who reported younger adults in the NHANES (1988 – 1994) data reported doing more outdoor physical activity than older adults. In their analysis, mean 25(OH)D levels decreased significantly with increasing age groups of 20 – 39 years, 40 – 49 years and ≥ 60 years (81). The higher prevalence of vitamin D supplements consumption among older age groups has been proposed by Tangpricha et. al. (2002) as a possibility for this 25(OH)D-age relationship (82).

In Europe the prevalence of 25(OH)D levels less than 10 ng/mL ranges between 2 – 30% among adults with lower rates recorded, surprisingly, in Northern Europe (83, 84). While the wide variation might be partly due to non-uniformity in assay methods, multi-center studies in Europe where 25(OH)D levels were assayed in a single laboratory,

higher serum 25(OH)D levels were still observed in Northern latitudes. This unexpected distribution of 25(OH)D concentrations between Northern and Southern European countries has been attributed to higher consumption of vitamin D rich fish, lighter skin pigmentation, and sun seeking behavior among Northern European populations (83, 84).

In Denmark a little more than half (52.2%) of the population aged between 30 and 60 years old was reported to have 25(OH)D levels < 20 ng/mL while 13.8% of the population had levels <10 ng/mL (85). In interpreting these data caution must be exercised because vitamin D intake from supplements was not accounted for in their analyses. The role of sedentary leisure time physical activity level was explored in this survey and found to be a strong predictor of 25(OH)D levels less than 20 ng/mL. However, interaction between leisure physical activity and age was not investigated which still leaves an explanation gap as to why the prevalence of vitamin D deficiency was greater in younger than older adults (< 65 years) in the NHANES data or in data from the UK's National Diet and Nutrition Survey (NDNS) 1992 – 2000. In the NDNS, the prevalence of 25(OH)D < 20 ng/mL peaked (75%) in the age group of 19 – 24 years then declined before peaking again among those 85 years and above (86).

Adequate vitamin D status in postmenopausal women is critical because of poor calcium metabolism associated with the postmenopausal period. Among 700 women aged between 60 and 80 years old referred to one of the osteoporosis referral centers across Italy, 27% of the subjects had 25(OH)D as low as less than 5 ng/mL while as many as 76% had 25(OH)D levels less than 12 ng/mL (87). Notwithstanding the inherent methodologic flaws associated with using data from clinical referral centers in this study,

selection bias related to targeting of high risk patients, and the fact that 25(OH)D measurements were taken at the end of winter season, this study still underscores the severity of vitamin D deficiency in populations of postmenopausal women.

In a study of the prevalence of vitamin D insufficiency among 8532 community-dwelling postmenopausal women, mean age 74.2 years, with osteoporosis or osteopenia across nine European countries, the prevalence of 25(OH)D levels less than 20 ng/mL was 32.1% (88). The prevalence was even higher, 45%, when the analysis was restricted to subjects under 65 years old. Even though the prevalence is relatively lower than what was reported by Isaia et. al. (2003) in Italy, this disparity could be explained by some of the factors associated with variable prevalence rates between European countries, as discussed earlier. Most importantly, the 8,532 women included in the analysis already had osteoporosis or osteopenia and hence may have already been taking vitamin D supplements. Holick et. al. (2005) reported even lower prevalence (18%) of 25(OH)D levels less than 20 ng/mL among North American postmenopausal women receiving osteoporosis therapy (77).

In summary, the prevalence of adult vitamin D deficiency, defined as either 25(OH)D < 20 ng/mL or < 10 ng/mL, is high worldwide in darker skinned populations, postmenopausal women, the elderly, and other nutritionally vulnerable populations. Several other risk factors predispose individuals and populations to low 25(OH)D concentrations. These factors and the prevention of deficiency D will be discussed in the text below.

1.3.4 Causes and Prevention of Adult Vitamin D Deficiency

Nutritional deficiency occurs when there is a disruption or malfunctioning in the synthesis of a nutrient from its endogenous or exogenous source. Endogenous synthesis of vitamin D from exposure to UVB radiation from the sun and diet are the major sources of vitamin D for humans – the rest is derived from vitamin D supplements (59, 68, 89, 90).

Exposure to sunlight at wavelengths in the UVB region (290 – 315 nm) is altered by several demographic, environmental and lifestyle factors. Lower latitude regions definitely have higher UVB radiation intensity compared to higher latitude regions. Indeed, ecological and within country (France) studies report relatively higher mean serum 25(OH)D concentrations in populations from regions close to the equator (83, 91). However, residence in lower latitudes don't always guarantee adequate serum vitamin D concentrations as demonstrated by multicenter studies in Europe where the prevalence of 25(OH)D levels less than 10 ng/mL was lower in populations residing in higher latitudes (84, 85). In temperate regions such as Europe, skin synthesis of vitamin D is optimized during the middle of the day (68). Hence the duration of outdoor activities, style of dressing, sunscreen use, and air pollution are all modifiable risk factors for low cutaneous synthesis of vitamin D (68, 86, 90). The non-modifiable risk factors are darker skin pigmentation and older age (> 65 years). The prevalence of vitamin D deficiency in darker skin populations is higher than in lighter skin populations. Even though the prevalence of low vitamin D may peak in younger adults (19 – 24 years) in the US, the prevalence rises after the age of 65 years for all racial groups (68, 79, 80, 86, 88, 90).

Low vitamin D consumption from foods and supplements is often related to socioeconomic, cultural, dietary lifestyles, and governmental nutritional policies (63, 79, 80, 82, 86, 92). For instance, it is mandatory in some countries, particularly developed countries, to fortify certain foods like milk, fruit juices, and cereals. These countries are also more likely to formulate public health programs for nutritionally vulnerable populations like children, pregnant and lactating women, and the very elderly. Populations that consume relatively higher quantities of oily fish are also more likely to be at lower risk for vitamin D deficiency.

Several disease conditions or malfunctioning in organs involved with the metabolism of vitamin D – liver and kidney – also increase the risk of vitamin D insufficiency. Individuals with liver failure lose the ability to synthesize adequate amounts of 25(OH)D (93, 94). Synthesis of $1,25(OH)_2D$ occurs primarily in the kidney, hence this function is lost in people with chronic kidney disease (93, 95). Decrease in the bioavailability of vitamin D can also occur in the presence of malabsorption conditions and obesity (96-98). In obese individuals, excess adipose fat tends to sequester vitamin D in the body thereby reducing its availability for normal biological functioning (94, 99, 100).

1.3.5 Prevention of Adult Vitamin D Deficiency

Setting the recommended daily allowance (RDA) for vitamin D has been as contentious as the definition of vitamin D deficiency in adults. This disagreement elicited responses from two major position documents for guidelines on the RDA for vitamin D from the Institute of Medicine and the Endocrine Society (61, 92). In 2010, the Institute

of Medicine (IOM) updated a previous report and recommended RDA values based on the IOM's conclusions that serum concentrations of 25(OH)D above 20 ng/mL were required for optimum bone health in diverse populations (74). The IOM recommended RDAs for individuals between 1 and 70 years of age of 600 IU/day and 800 IU/day for those above 70 years old. While the Endocrine Society Clinical Practice Guideline agrees with the recommendations put forth by the IOM, they made revisions based on, in addition to bone health, muscle functioning and 25(OH)D levels above 30 ng/mL. The Endocrine Society proposed minimum supplementation of at least 1,000 IU/day and 1,500 – 2,000 IU/day to achieve minimum serum 25(OH)D > 30 ng/mL for age groups 1 – 18 years, and 19 + years respectively. The Endocrine Society guidelines are for "at risk" clinical populations, however, as opposed to the IOM's public health recommendations for generally healthy populations. Bischoff-Ferrari et. al. (2006) recommended consumption of 1000 IU/d vitamin D to achieve serum concentrations of 30 ng/mL in at least 50% of the adult population (101). These reports also recommended vitamin D daily intakes for the prevention of some muscoloskeletal disorders and colorectal cancer. Lastly, the IOM also proposed tolerable upper level intakes of 4,000 IU/day, 3,000 IU/day and 2,500 IU/day for 9+, 4 – 8, and 1 – 3 year groups respectively (74).

1.3.6 Consequences of Low Vitamin D Status on Non-musculoskeletal Conditions Among Adults

The relationship between low vitamin D status and several non-musculoskeletal diseases in humans has also been controversial. Mortality data reflect the overall health

status of a population; hence, the association between vitamin D status and mortality has been extensively studied (102-106).

1.3.7 Vitamin D and Mortality

Observational studies

A meta-analysis of 13 observational studies reported a 42% increase in risk of all-cause mortality (RR = 1.42, 95%CI: 1.23-1.63) associated with the lowest category of serum 25(OH)D compared to the highest (or referent category) (102). Even though the authors complied with most of PRISMA's (Preferred Reporting Items for Systematic reviews and Meta-analysis) guidelines, some methodological issues still need to be addressed. First, most of the 13 studies were poor in quality because they were not originally designed to test the association between serum 25(OH)D levels and mortality. For example, one of the studies that contributed most of the sample (13%) had a very short follow-up period of just 1.3 years (103). Reverse causality could not be ruled out in such a short study. This study also failed to adjust for physical activity, BMI, and smoking status which could have confounded the reported effect estimate of HR = 1.77 (95% CI: 1.49 – 2.10). The largest study by Melamed et. al. (2008) which contributed 13.8% of the analytical sample had long follow-up periods between 6 – 12 years and adjusted for pertinent confounders (104). A significant risk (HR) of 1.28 (95% CI: 1.28 – 1.48) was still observed for all-cause mortality, albeit relatively weaker. Lastly, there were inconsistencies in the definition of vitamin D deficiency based on serum 25(OH)D levels between the 13 studies reviewed. Specifically, most of the studies used cut-off values below the recommended 20 ng/mL (50 nmol/mL) levels for vitamin D

insufficiency. Accordingly, the author posited the mortality risk observed in their meta-analysis was underestimated (102).

Other large observational studies in adult populations have also reported increased risk of all-cause mortality with low serum 25(OH)D levels (105, 106). Among a prospective cohort of 3,258 subjects with mean age 62±10 years referred for coronary angiography at a single center in Germany, the risk of mortality associated with the lower quartile of 25(OH)D was about 67% higher compared to those in the upper quartile. For the two lower quartiles with median 25(OH)D concentrations of 7.6 ng/mL and 13.3 ng/mL respectively, the associated HR were 2.08 (95% CI: 1.60 – 2.70) and 1.53 (95% CI: 1.17 – 2.01) respectively. The cardiovascular mortality risk associated with these same lower quartiles of serum 25(OH)D levels were 2.22 (95%CI: 1.57 – 3.13) and 1.82 (95%CI: 1.29 – 2.58) respectively (105). However, caution has to be exercised when interpreting the CVD mortality risk in this population because of the high all-cause mortality rate (22.6% for 7.7 years median follow up) in this population. The high all-cause mortality rate is indicative of the relatively poor health status of this population which could have been a residual confounder for the 25(OH)D-CVD mortality association.

In the general USA adult population, 2001 – 2004 NHANES, the risk of all-cause mortality associated with 25(O)D levels was not strong. In multivariable analysis, the risk (HR) of all-cause mortality among those with 25(OH)D < 20 ng/mL compared with those with ≥ 30 ng/mL was 1.28 (95% CI: 0.86 – 1.90) (106). A drawback to this analysis lies

with the relatively short median follow-up time of 3.8 years for mortality in a relatively young and healthy population of 7,531.

In appraising existing evidence, observational studies support an inverse association between serum 25(OH)D concentrations and all-cause mortality. The next scientifically logical step is to investigate the effect of vitamin D supplementation and mortality in randomized controlled trials to rule out the possibility of bias and reverse causality. Several randomized trials of vitamin D have been conducted among primary prevention populations to test its effect on fractures in older adults. Data from such studies have also been analyzed to test the effect of vitamin D supplementation (alone or combined with calcium) on all cause mortality.

Randomized controlled trials (RCTs)

Two different meta-analyses of vitamin D RCTs concluded that vitamin D supplementation was protective against all-cause mortality. In the first published meta-analysis by Autier and Gandini (2006), 18 RCTs of vitamin D (D_2 or D_3) published before 2007 involved a total sample of 57, 311 with 4,777 deaths and a mean 5.7 years period. A 7% reduction in all-cause mortality risk (RR = 0.93, 95%CI: 0.87 – 0.99) was achieved with supplementation of a trial size-adjusted mean daily vitamin D dose of 528 IU (107). A few observations should be considered when interpreting the results from this meta-analysis. First, none of the 18 trials in the meta-analysis pre-specified mortality as their primary outcome. Second, while most of these trials were conducted in older ($\geq$ 64 yrs) populations who are at increased risk of fractures, data from the WHI vitamin D and

calcium trial (N = 36,282), which contributed half of the analysis population, included relatively younger females (108).

Findings of the meta-analysis by Autier and Gandini (2006) have been confirmed by the most extensive meta-analysis on vitamin D supplementation and all-cause mortality (7). Bjelakovic et. al. (2011) conducted exhaustive searches of all major databases as well as consultation with experts and pharmaceutical companies for additional data. Fifty published randomized trials of supplemental vitamin D (D_3 or D_2), or its active forms (calcitriol or alfacalcidol) were included in the analysis – 32 of these trials combined supplemental vitamin D with calcium. The majority (44/50) of these trials were primary prevention trials with a total of 93,585 subjects that included healthy volunteers, postmenopausal women, and the elderly. The relative risk (RR) of all-cause mortality associated with total vitamin D supplementation with a median daily intake of 800 IU, 1,000 IU, 1 µg, and 0.5 µg of D_3, D_2, alfacalcidol, and calcitriol respectively and calcium (among 32 trials) was 0.97 (95% CI: 0.94 – 1.00). When stratified on the quality of trials reviewed, the risk estimate was slightly reduced and remained significant, 0.95 (95% CI: 0.91 – 0.99), among 26 low bias studies, but was slightly higher and not significant among 24 high bias studies. Compared to the other vitamin D analogues that were used in the trials, D_3 was the only one observed to be significantly associated with reduced risk of all-cause mortality, 0.94 (95% CI:0.91 – 0.98). The authors also emphasized the importance of knowing the baseline vitamin D status of trial participants by showing that the intervention was only effective among participants with vitamin D insufficiency at baseline. The analysis also reported that vitamin D significantly reduced the risk of death among only participants who received dosages less than 800 IU/day.

This supports a proposition by Shapses and Manson, IOM committee members, that "more is not necessarily better" (109).

Only a small reduction, 3%, in all-cause mortality was achieved through supplementation, however, this study had sufficient statistical power to detect such a small effect estimate. The follow-up periods for most of these 50 trials were relatively short – 39 of the 50 trials had follow-up years of 3 years or less. Other important lessons were learned from this large meta-analysis. While vitamin D significantly decreased the risk (RR = 0.95, 95% CI: 0.91 – 0.99) of mortality for participants with 25(OH)D $\leq$ 20 ng/mL at baseline, there was no benefit for those already with 25(OH)D $\geq$ 20 ng/mL vitamin D status at baseline.

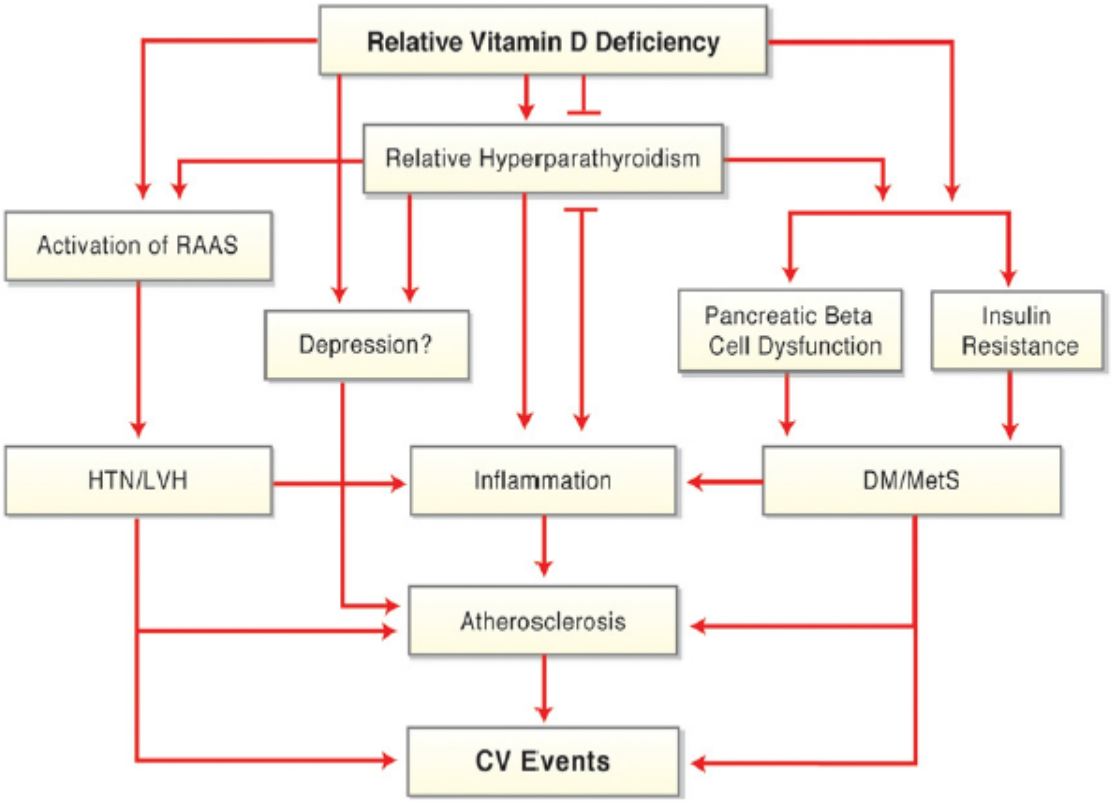
A drawback to the meta-analysis studies of both observational and randomized trials is that some of the studies did not have enough long follow-up periods to fully assess the effect of the supplementation. The short durations of follow-up also makes it difficult to rule out mortality due to preexisting conditions. However this temporality effect would have been similar in both intervention and control groups for RCTs. In conclusion, it appears vitamin D supplementation significantly reduced the risk of all-cause mortality by modest magnitude. Furthermore, low serum 25(OH)D levels also predicted higher risk of all-cause mortality.

CHAPTER 2

EVIDENCE FOR ASSOCIATION BETWEEN VITAMIN D DEFICIENCY AND HEART FAILURE

2.1 Proposed Mechanisms by Which Vitamin D may Reduce CVD Risk

The ubiquity of vitamin D receptors in human tissues including cardiac muscle has stimulated research on the pathophysiological role of vitamin D in CVD and HF pathogenesis. Several plausible mechanisms have been proposed. These mechanisms suggest vitamin D may affect cardiometabolic function through intermediate pathways or systems, Figure 2 (110). Pathophysiological evidence in human studies, however, is limited, and most data are derived from animal studies.



CV = cardiovascular; DM = diabetes mellitus; HTN = hypertension; LVH = left ventricular hypertrophy; MetS = metabolic syndrome; RAAS = renin-angiotensin-aldosterone system.

Figure 2. Potential mechanisms for cardiovascular effects of vitamin D deficiency

The renin-angiotensin-aldosterone system (RAAS) plays a major role in HF pathogenesis by regulating blood pressure, cardiac contractility, electrolyte homeostasis, and eccentric hypertrophy of the myocardium (111, 112). It has been demonstrated that mice without the vitamin D-receptor (VDR) developed high blood pressure, cardiac enlargement and experience increased activation of the RAAS (113). The activation of RAAS by low vitamin D status has recently been noted in human studies (114, 115). Among healthy normotensive subjects, Forman et. al. (2010), observed vitamin D deficiency (25[OH]D < 15 ng/mL) to be associated with elevated levels of angiotensin II (ang II) as well as blunted plasma renin flow (114). In another study, by Tomaschitz et. al. (2010). Both 25(OH)D and 1,25$(OH)_2$D levels were identified as independent predictors of plasma renin concentrations (PRC) and Angiotensin II levels among patients referred for coronary angiography; decreasing levels of both vitamin D markers were correlated with higher PRC and Ang II levels (115). Lower 25(OH)D concentrations had already been previously shown to be associated with increased risk of stroke and heart failure mortality in this population (105, 116, 117). This suggests a strong plausible role of vitamin D in regulating the RAAS in HF pathogenesis.

Hyperparathyroidism has also been postulated as an intermediate process in vitamin D-heart failure pathophysiology. Evidence for this derives from studies in patients with end-stage renal disease (ESRD) (14-17). ESRD patients lose the ability to convert 25(OH)D to the metabolic form of 1,25$(OH)D_{2,}$ leading to secondary hyperparathyroidism and elevation of circulating parathyroid hormone (PTH) levels (10, 15-17). Chronic exposure to PTH has been reported to be associated with abnormal myocardial structure and functioning as well as elevated blood pressure and accelerated

atherosclerosis. Excess PTH may also lead to cardiomyocyte hypertrophy which is an important risk factor for HF and especially among hemodialysis patients (13, 15, 118).

A pathophysiologic model of a direct vitamin D effect on cardiac function has not been extensively studied in humans. However, data from animal models suggest that vitamin D may regulate cardiac functions via interaction with vitamin D receptors (VDR) in cardiac myocytes (10, 119). Vitamin D ($1,25[OH]_2D$) regulates intracellular calcium homeostasis and calcium ion uptake in ventricular cardiac muscle cells through a receptor-mediated mechanism to modify cardiac contractility (120). To demonstrate this, physiological functioning markers in VDR-knockout mice and wild type mice were compared through histological staining of their cardiac tissues after 12 months of life. Poor physiological cardiac functioning markers, cardiac hypertrophy, cardiac fibrosis, collagen deposition, were all observed in the VDR-knockout mice but not in the wild type mice (11, 12).

Evidence from both human and animal studies suggests vitamin D regulates cardiac functioning via direct and indirect routes. Vitamin D can directly affect cardiac functioning through interaction with cardiomyocyte VDR or indirectly by modifying the major risk factors associated with HF.

2.2 Association between Low Vitamin D Exposure and Heart Failure

However, only two published observational studies have investigated a direct relationship between vitamin D deficiency and HF incidence among a cohort free of heart failure at baseline (103, 116). Additional data on this topic are derived from clinical trials

of vitamin D supplements among prevalent HF cases (121-123). These studies are discussed.

RCT data among HF patients

There is an absence of RCTs of the primary prevention role of vitamin D in HF. However, secondary prevention trials of HF outcomes through vitamin D supplementation are reviewed; secondary prevention data are essential in understanding the pathophysiological role of an intervention in the pathogenesis of a disease. In Germany, 123 middle-aged (50 – 63 years) HF patients were randomized to treatment with daily doses of 2000 IU cholecalciferol and 500 mg Ca or to 500 mg calcium only (control) for nine months (121). Statistically significant reductions in mean parathyroid hormone levels and elevation in Interleukin-10 levels were observed in the treatment group but not in control group. Additionally, tumor necrosis factor-α levels remained unchanged in the treatment group but increased in the control group suggesting that vitamin D suppresses this marker of inflammation. However, vitamin D supplementation had no observed effect on left ventricular ejection fraction or N-terminal fragment brain natriuretic peptide (NT-proBNP) levels. This trial suggests vitamin D may be associated with positive changes in the anti-inflamatory milieu of HF patients but may not improve ejection fraction once disease is established.

Results of other studies have been inconsistent. Among 69 elderly HF patients (mean age, 79 years), supplementation (with 100,000 IU of ergocalciferol) resulted in significant reduction in NT-proBNP levels (secondary outcome) after 20 weeks, -52 pg/mL, $p = 0.05$, but did not improve physical functioning or quality of life (122). Another study, among African Americans (n = 14), reported attenuation of

hyperparathyroidism, oxidative stress, and improvement in ventricular function after a fourteen week regimen of vitamin D and calcium supplementation (123).

Observational cohort studies

Results from observational studies also suggest an association between low vitamin D status and HF. Very low 25(OH)D levels (≤15ng/mL) were associated with a two-fold increase in HF incidence (HR=2.01, $p < 0.0001$) in a population of 23,793 middle-aged (≥ 50yrs) subjects derived from a database of electronic medical records (103). A prospective study among 3,299 patients in Germany, free of HF and referred for coronary angiography, reported inverse associations between 25(OH)D and $1,25(OH)_2D$ levels and NT-proBNP levels ($R = 0.190$; $P < 0.001$). Both vitamin D metabolites were positively correlated with left ventricular function measured with contrast ventriculography ($P < 0.001$ for both). Furthermore, persons with 25(OH)D levels within the lowest quartile were at elevated risk of HF mortality, HR=2.64, 95%CI (1.47– 4.72), compared to those in the highest quartile (116).

2.3 Association between low vitamin D exposure and major HF risk factors

There is a moderate but inconsistent body of evidence to support inverse associations between vitamin D levels and major cardiovascular precursors of HF - hypertension, diabetes, and cardiovascular diseases (especially myocardial infarction, angina, and valvular heart disease) (19). The American College of Cardiology (ACC) classifies individuals with hypertension, cardiovascular diseases, coronary heart diseases/events, or diabetes but who have not yet developed structural abnormalities like left ventricular dysfunction or hypertrophy or geometric chamber distortion as 'Stage A'

heart failure and those who demonstrate any of these structural abnormalities as 'Stage B'. While Stages A and B heart failure are not HF per se, these designations enable healthcare providers to identify individuals at the greatest risk of HF for early interventions (18). In the general female population between the ages of 40 – 89 years, hypertension, diabetes, and CVDs are associated with population attributable risks (for HF) of 59%, 12%, and 26%, respectively (19). Interestingly, these precursors of HF have also been found to be inversely correlated with serum vitamin D levels (5-9). Hence, these cardiovascular risk factors can potentially modify the vitamin D-HF relationship – this supports the indirect effect of vitamin D on HF model. Hence, if vitamin D supplementation can reduce the prevalence or improve the prognosis of these risk factors, then HF risk may be curtailed. It is therefore important to evaluate the role of vitamin D, with/without calcium, in both incident and prevalent cohorts of cardiovascular disease risk factors.

A prevalent cohort has been described as a study population with risk factors for the outcome of interest and/or the outcome of interest during a specific period of time. In contrast, an incident cohort consists of a population free of both the risk factors and/or the outcome at the inception of the cohort but who are at risk of developing the risk factors and/or outcome during the study period (124). Prevalent and incident cohorts tend to differ considerably on baseline and prognostic characteristics of the disease of interest (124). To demonstrate this phenomenon, Buckley et. al. (2010) evaluated survival in both prevalent (first acute myocardial infarction) and incident cohorts derived from primary care and death certificate linked data. During a five year period, there were more deaths (from all-cause [34%] and ischemeic heart disease, IHD [18.5%]) in the prevalent cohort

than in the incident cohort (all-cause [18%] and IHD [12.2%]). This is why it is important to evaluate the effect of the intervention, calcium plus vitamin D, in the vitamin D and calcium trial of the WHI study in both incident and prevalent cohorts. The associations between vitamin D and known strong risk factors of HF are reviewed below.

2.3.1 Cardiovascular Diseases (CVD)

Evidence from RCTs

The association between vitamin D alone or combined with calcium and the risk of CVD incidence is equivocal and based on sparse RCT data. Wang et. al. (2010) systematically reviewed and conducted a meta-analysis of RCT data on vitamin D and calcium supplementation and the risk of CVD events (5). On calculating a pooled RR for the association between vitamin D alone and CVD incidence assessed as a secondary outcome, only two studies met the inclusion criteria for the review - Trivedi et. al. (2003) and Prince et. al. (2008) (5, 125, 126). The study by Trivedi et. al. (2003) contributed 90% of the analytical sample, supplemented 100,000 IU of oral vitamin D_3 per every four months (an average of ~833 IU/day) to a placebo matched group (1,019 men and 326 women) community-dwelling population of 65 years and above (total N = 2,686) in Britain. The risk of composite CVD - ischemic heart disease (ICD-9; 410.0 - 414.0) and cerebrovascular disease (ICD-9; 430.0 - 438.0) - assessed as a secondary outcome was not significantly reduced (RR = 0.84, 95% CI: 0.65 – 1.10). It is also not clear if administering oral vitamin D doses as high as 100,000 IU, above the IOM daily threshold, every four months would have the same effect on CVD incidence as supplementing daily doses within the recommended range. Given that Bjelakovic et. al.

(2011) found daily vitamin D_3 but not intermittent supplementation to be associated with reduction of all-cause mortality, it is possible that daily supplementation is more beneficial for CVD outcomes as well (7).

In the vitamin D combined with calcium pooled analysis, 99% of the analytical sample came from the WHI trial while only 1% came from the other study, by Brazier et. al. (2005) (5, 127, 128). The WHI intervention arm was supplemented with only 400 IU/day of vitamin D combined with 1000 mg/day of calcium. This amount was lower than the revised recommended daily intake of 600 IU/day by the IOM for this age group, which included diet plus supplements (92).

Vitamin D (alone or combined with calcium) supplementation had no effect on myocardial infarction, stroke, or ischemic heart disease when investigated as individual fatal or non-fatal CVD endpoints. When Trivedi et. al. (2003) stratified their analysis by separate CVD endpoints, supplementation neither reduced the risk of ischemic heart disease (ICD-9; 410.0 - 414.0) (RR = 0.94, 95% CI: 0.77 – 1.15) nor ischemic heart disease mortality (RR = 0.84, 95% CI: 0.56 – 1.27) (125). In the WHI study, calcium/vitamin D supplementation had no benefit on MI or stroke risk while in a Another study based on the WHI data reported the intervention had no significant effect on coronary heart disease (HR=1.01, 95%CI: 0.79 – 1.29) or cerebrovascular disease mortality (HR=0.89, 95%CI: 0.62 – 1.29) (128, 129).

Observational cohort studies

The vitamin D – CVD association has been inconsistent in cohort studies. The Framingham Offspring Study investigated CVD events as composite endpoint. Among

1,739 men and women in this study (mean age 59 years), CVD incidence decreased in a graded manner with increasing categories of 25(OH)D levels (p-trend = 0.01) but appeared to plateau at levels around 24 ng/mL (117). Compared to 25(OH)D ≥ 15 ng/mL, the risk of CVD incidence associated with levels < 15 ng/mL and < 10 ng/mL were 1.59 (95% CI:1.03 – 2.45) and 1.81 (95%CI:1.03 – 3.18) respectively (130). Total CVD mortality risk on the other hand was inversely associated with 25(OH)D concentrations in two (Dobnig et. al. [2008] and Kilkkinen et. al. [2009]) of the three studies selected for a systematic review by Pittas et. al. (2010) (6). Dobnig et. al. (2008) reported the lower two quartiles of 25(OH)D levels, 7.6 ng/mL and 13.3 ng/mL, to significantly predict elevated risk of CVD mortality; 2.22 (95% CI: 1.57 – 3.13) and 1.82 (95% CI: 1.29 – 2.58) respectively for the first (Q_1) and second (Q_2) lower quartiles (105). Reduced risk of total CVD mortality (HR = 0.76, 95% CI: 0.60 – 0.95) was associated with the highest quintile of 25(OH)D compared to the lowest in the Mini-Finland Health Survey by Kilkkinen et. al. (2009). This study prospectively followed 6,219 participants aged 30 years and older who were free of baseline CVD, for a period of (mean) 27 years in Finland (131). The third study, based on NHANES III data, did not find a significant association between 25(OH)D levels and total CVD mortality (104). The systematic review by Pittas et. al. (2010) is a good reported because it was conucted in accordance with factors from recommended standardized forms of reporting, such as the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) and CONSORT (Consolidated Standards of Reporting Trials) (132, 133). They did not meta-analyze the three studies because of the heterogeneity in the definition of total CVD mortality between the studies.

Myocardial infarction (MI) is the most consistent cardiovascular event contributing to the incidence of HF (19). The association between MI and low vitamin D status is inconclusive. Only one of the two studies included in the systematic review by Pittas et. al. (2010) reported an association between MI and low vitamin D status (6). In the Health Professionals Follow-up study, very low 25(OH)D serum concentrations (≤ 15 ng/mL) were associated with a two-fold increased risk of MI, HR = 2.09 (95% CI:1.24 – 3.54), compared to 25(OH)D ≥ 30 ng/mL (134). The other study failed to report the specific 25(OH)D levels that were compared for association with MI – they only alluded to using the lowest quartile as the referent category. The authors also failed to specify which biomarker of vitamin D was used in their analysis as they measured both 25(OH)D and $1,25(OH)_2D$ concentrations (135).

2.3.2 Diabetes

The association between diabetes and vitamin D insufficiency is inconclusive. Niether RCT nor cohort studies data provide sufficient evidence for a causal association between vitamin D deficiency and type 2 diabetes. However, vitamin D deficiency might still have a strong effect on prediabetes, which is a strong risk factor for HF.

Evidence from RCTs

Vitamin D supplementation had no significant effect on fasting plasma glucose level among a population with normal glucose tolerance at baseline in a meta-analysis of 5 trials (6). However, one trial conducted a sensitivity analysis among a population with impaired glucose tolerance at baseline (n = 222) and observed attenuation in increasing fasting plasma glycemia through vitamin D (700 IU) and calcium (500 mg)

supplementation (136). Recent data from the Randomized Placebo-Controlled Trial of Vitamin D3 and/or Calcium (RECORD) trial among 5,292 participants aged 70 years and above did not observe a significant effect of vitamin D (800 IU) supplementation alone or with 1000 mg calcium (137). Vitamin D and calcium supplementation did not achieve a significant reduction of the risk of type 2 diabetes, OR = 0.68 (95% CI:0.40 – 1.16) among participants who complied with the treatment protocol after the second year of the study, (137).

Prospective cohort studies

Only a few large prospective cohort studies have evaluated the association between vitamin D status and type 2 diabetes. Of four studies that met the inclusion criteria for a systematic analysis by Pittas et. al. (2010), only the Women's Health Study reported a significant association between vitamin D deficiency and the risk of type 2 diabetes (6). The Women's Health Study defined vitamin D deficiency based on self-reported total vitamin D intake instead of the more sensitive biomarker, 25(OH)D, which limits their findings (138). Another of the four reviewed studies which also poorly assessed vitamin D deficiency by this total vitamin D intake method did not find significant association between low vitamin D intake and risk of type 2 diabetes, HR = 0.87 (95% CI:0.69 – 1.09) (139). Data from a large medical records database (n = 41,504) demonstrated that very low vitamin D status (25[OH]D ≤ 15 ng/mL) was associated with increased incidence of diabetes mellitus compared to normal vitamin D status (25[OH]D > 30 ng/mL) (103).

The lack of observed strong associations between vitamin D and risk of type 2 diabetes could be partly explained by study design issues. First, all of the studies reviewed here ascertained diabetes cases through self-reports. While some reported cases were validated, none of the studies screened the study population for diabetes or re-adjudicated these cases to minimize misclassification bias. Second, most of the RCTs had small samples and short duration of follow-up. The observational prospective cohort studies also introduced misclassification errors into the ascertainment of vitamin D status by using total vitamin D intake rather than 25(OH)D levels . Lastly, in addition to the misclassification issue, it is possible that some participants were asymptomatic but were pre-diabetic. To buttress this point, a recent publication of the NHANES III data reported an association between low serum 25(OH)D levels and risk of pre-diabetes, OR = 1.47 (95% CI:1.16 – 1.85). The authors of this study defined pre-diabetes as "a 2-h glucose concentration of 140–199 mg/dL, or a fasting glucose concentration of 110–125 mg/dL, or an A1C value of 5.7–6.4%" among a sample of adults aged 20 years and over who were free of diabetes and CVD (140).

2.3.3 Hypertension

One of the strongest biologically plausible benefits of vitamin D is its role in the up- regulation of RAAS. It is hypothesized that vitamin D regulates blood pressure via the RAAS. This hypothesis, however, has been investigated in both observational and RCTs with equivocal conclusions.

Evidence from RCTs

The findings regarding the effect of vitamin D alone or combined with calcium on blood pressure has been inconsistent. The authors of the AHRQ systematic review of health outcomes associated with vitamin D and calcium did not meta-analyze selected trials because of the heterogeneity in study populations and follow-up periods (8). However, a recent meta-analysis of 10 trials, Pittas et. al. (2010), reported no significant reduction in either systolic nor diastolic blood pressures as a result of vitamin D supplementation (6). The conclusion of this analysis warrants caution due to several factors. First, based on their own assessment of the quality of these 10 trials, the authors judged only 3 to be of "good quality". Nevertheless, they pooled effect estimates from all 10 trials. Second, 9 of the 10 trials had very short follow-up periods, 5 – 52 weeks. The notable exception is the WHI study with 7 years follow-up. WHI contributed about 97.6% (36,282) of the analytical sample for the meta-analysis (141). The WHI trial did not find significant effect of combined vitamin D and calcium supplementation on either systolic or diastolic blood pressure. Inherent study design issues such as the low vitamin D dosage could have played prominent role in the outcome. Lastly, there was significant heterogeneity between the studies, $I^2 = 69\%$, which raises concerns about the validity of conducting the meta-analysis.

Observational cohort studies

Pittas et. al. (2010) meta-analyzed 3 studies from two cohorts and reported a 76% increase in risk of hypertension (RR=1.76, 95%CI: 1.27 – 2.44) associated with the lowest level of 25(OH)D (< 14.8 to 20.4 ng/dL) compared to the highest level (>30 to

32.4 ng/dL) after 7 to 8 years of follow-up (6, 142, 143). However, among 1,206 adults (≥ 65years) in the Netherlands, low vitamin D level (25[OH]D < 25nmol/L) was not associated with hypertension (OR = 0.89, 95% CI: 0.47 – 1.69) (144). The prevalence of vitamin D insufficiency, (25[OH]D < 20 ng/mL), 10.5%, was relatively low in this elderly population, however. This could have been partly due to vitamin D supplement intake, which the study did not assess or adjust for in their model.

CHAPTER 3

METHODOLOGY

3.1 Objectives

3.1.1 Main Objective

The main objective of this study is to determine if supplementation with vitamin D plus calcium reduced the incidence of hospitalization for heart failure (HF) among postmenopausal women. Heart failure caries enormous healthcare cost, mortality, and functional limitation yet there are inadequate preventive measures against HF at the population level. The data reviewed above support the hypothesis that supplementation with vitamin D among primary prevention cohorts might be protective against HF incidence. However, there are no known published randomized trials that have investigated such intervention for the prevention of HF. This study therefore seeks to fill that knowledge gap by analyzing data from the vitamin D and calcium trial of the Women's Health Initiative (WHI) study. The WHI is an ongoing long-term health study of postmenopausal women focused on preventing chronic diseases including heart failure. The study was designed in two parts consisting of three clinical trials and an observational study. The vitamin D and calcium (CaD) trial is one of the three clinical trials nested within WHI, and was primarily designed to test the effect of vitamin D and calcium intervention for the prevention of osteoporotic fractures and colorectal cancer. However, heart diseases, including hospitalized heart failure, were assessed as secondary

outcomes. This national population-based study is ideal for investigating the main objective of this study.

3.1.2 Specific Aims

Specifically, the following aims will be evaluated to assess the impact of vitamin D plus calcium supplementation on the incidence of hospitalization for HF.

i. To evaluate whether supplementation with vitamin D plus calcium is modified by baseline risk status of heart failure. It is hypothesized that vitamin D may protect against HF indirectly via minimizing the incidence and/or progression of major HF risk factors.

ii. To assess whether self-reported total vitamin D and calcium intake at baseline modified the association between the intervention and HF incidence. Participants who reported adequate vitamin D intake may not benefit from this intervention because of their optimal vitamin D status as previously observed (7).

By accomplishing the above objectives, this study will help explain the pathophysiological role of vitamin D in heart failure pathogenesis. Additionally, results of this study, if they demonstrate benefit, will be useful for design and implementation of public health intervention against HF through vitamin D supplementation. Clinically, the study may also help to inform clinical decision making about the utility of screening high risk HF individuals for vitamin D deficiency.

3.2 Hypotheses

Vitamin D plus calcium supplementation is associated with a lower risk of heart failure. Populations that may be at relatively higher predisposition for developing heart failure, owing to the presence of preexisting cardiovascular risk factors of heart failure (stages A or B heart failure), will benefit more from this intervention compared to populations without any preexisting baseline cardiovascular risk factors of heart failure.

Primary Hypothesis 1

Research hypothesis (H_1): Vitamin D (400 IU/day) plus calcium (1000 mg/day) supplementation was associated with lower risk of heart failure during an average of 7.13 (SD, 1.33) years of follow-up.

Null hypothesis (H_0): Vitamin D (400 IU/day) plus calcium (1000 mg/day) was not associated with risk of heart failure after randomization to vitamin D and calcium.

Primary Hypothesis 2

Preexisting baseline cardiovascular risk factors of heart failure modifies the association between the intervention and heart failure incidence. While the intervention is associated with lower risk of heart failure in both populations stratified by preexisting baseline cardiovascular risk factors of heart failure, the effect estimate for this association is higher in populations with these risk factors.

Research hypothesis (H_{2a}): Calcium (1000 mg/day) plus vitamin D (400 IU/day) supplementation was associated with lower risk of heart failure in populations with preexisting baseline cardiovascular risk factors of heart failure during an average of 7.13 (SD, 1.33) years of follow-up.

Null hypothesis (H_0): The intervention is not associated with risk of heart failure after randomization to vitamin D and calcium in this population.

Research hypothesis (H_{2b}): Calcium (1000 mg/day) plus vitamin D (400 IU/day) supplementation was associated with lower risk of heart failure in populations without preexisting baseline cardiovascular risk factors of heart failure during an average of 7.13 (SD, 1.33) years of follow-up.

Null hypothesis (H_0): The intervention is not associated with risk of heart failure after randomization to vitamin D and calcium in this population.

Secondary Hypothesis

Participants were allowed to continue the consumption of personal calcium (≤ 1,200 mg/day) and vitamin D (≤ 600 IU/day) supplements while enrolled in this trial due to ethical reasons. Hence, total (from diet and supplements) vitamin D and calcium intakes may have modified the associations between the intervention and heart failure incidence.

Research hypothesis (H_{3a}): Baseline self-reported total vitamin D intake modified the association between the intervention and risk heart failure incidence.

Null hypothesis (H_0): Baseline self-reported total vitamin D intake did not modify the association between the intervention and heart failure incidence after randomization to vitamin D and calcium.

Research hypothesis (H_{3b}): Baseline self-reported total calcium intake modified the association between the intervention and risk heart failure incidence.

Null hypothesis (H_0): Baseline self-reported total calcium intake did not modify the association between the intervention and heart failure incidence after randomization to vitamin D and calcium.

3.3 Overview of the WHI study

In 1991, the National Institute of Health established the Women's Health Initiative (WHI) study. The initiation of this study was aided by strong public interest in women's health backed by political will and interests from the scientific community to identify preventive strategies for colorectal and breast cancers, cardiovascular diseases, and osteoporotic fractures (145). The WHI study with 161,000 participants and a financial budget of $628 million for fifteen-year period makes it the largest of its kind (146).

A Clinical Coordinating Center (CCC) was established to develop drafts of the study protocol and collect, manage and analyze data. Additionally, forty Clinical Centers (CC) were established across the United States of America to recruit and enroll participants into the WHI study. WHI began enrolling participants in the Fall of 1993. This large study of postmenopausal women consisted of three clinical trials (CT) and one observational study. Initially, women recruited into the study had the choice to enroll in either the Hormone Therapy (HT) or Dietary Modification (DM) trials or both. After a year of two, participants of these two trials were invited to join the Calcium plus Vitamin D (CaD) trial. Women who did not join any of the three trials were invited to be part of the observational study. These studies are described later in this chapter. Participants were followed through to 2005 to close out the trials and the observational study.

However, the WHI study was extended two more times, 2005 – 2010 and 2010 – 2015 to continue collecting follow-up data on consenting participants.

Details of the WHI study design and implementation have already been published (146, 147). More than 161,000 women (50 – 79 years old) were recruited for the study making it the largest study among postmenopausal women. Postmenopausal period was defined as no menstrual period for at least 6 months if ≥ 55 years or absence of menstrual period for a period of at least 12 months if between 50 and 54 years old. Three clinical trials (Dietary Modification, Hormone Therapy, and Vitamin D and calcium) and an observational study were designed to assess prevention therapies for diseases and the overall health and well-being of postmenopausal women (146, 147). A total of 68,132 postmenopausal women in the age groups of 50 – 54 years (10%), 55 – 59 years (20%), 60 – 69 years (45%), and 70 – 79 years (25%) were recruited at 40 clinical sites over the U.S. and randomized to each of the three clinical trials (CT). There was considerable overlap of participants between the three CTs as depicted in Figure 3 (147).

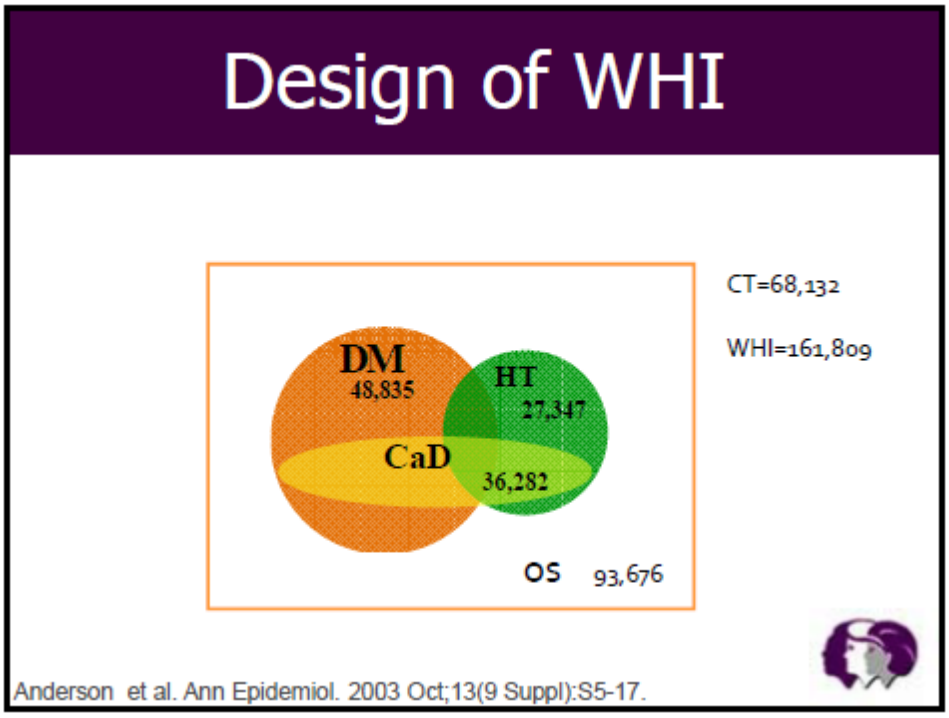


Figure 3. Overlap of participants enrolled in the three clinical trials (HT, DM, and CaD) of the Women's Health Initiative (WHI) study.

Dietary Modification (DM) Trial

The dietary modification (DM) trial was designed to assess the impact of a low fat dietary pattern on colorectal and breast cancers as major primary outcomes and CVDs as secondary outcomes. Of the 48,435 individuals recruited into the DM trial, 40% were randomized to a sustained low fat dietary eating pattern while 60% were randomized to the control group to maintain a self-selected dietary behavior. The intervention arm was designed to achieve a 20% reduction in total dietary fat and 7% reduction of saturated fat intake per corresponding total caloric intake. The secondary objectives of the intervention were to increase daily vegetable and fruits intake to six and grain products intake to five. Further details of the DM trial are published elsewhere (146, 148).

Hormone Therapy (HT) Trial

The Hormone Therapy (HT) trial design, implementation, and safety concerns about the intervention have been published (149-151). The HT recruited 27,500 postmenopausal women for a double-blind 1:1 randomized study on the effects of two forms of estrogen on coronary heart disease (CHD). The form of the estrogen was dependent on the hysterectomy status of participants during randomization. Hip and other bone fractures were assessed as secondary outcomes while breast cancer was investigated as an adverse outcome. At randomization, post-hysterectomy women (n=10,739) were randomized to unopposed estrogen therapy of conjugated equine estrogen (E alone) 0.625 mg/day or placebo while women with an intact uterus (n=16,068) were randomized to the same preparation of estrogen plus continuous 2.5 mg/day of medroxyprogesterone acetate (E + P) daily or placebo. In the year 2002, the E + P trial was stopped prematurely by the WHI Data and Safety Monitoring Board (DSMB) owing to accumulated evidence that E + P was associated with increased risk of breast cancer and CVD. It was also observed that the overall risk-to-benefit ratio of the intervention was elevated during this period. Subsequently, in 2004 the E alone trial was also stopped by the DSMB after accumulated evidence of increased risk of stroke and no benefit for CHD (152).

3.4 The Calcium plus Vitamin D (CaD) Trial

The study design and methodology of the CaD study has already been published (147, 153). Cardiovascular diseases, colorectal and breast cancer, and osteoporotic fractures were identified as the most important health outcomes in older women. The vitamin D and calcium (CaD) trial was initiated as the last of the three trials to primarily

evaluate the impact of vitamin D and calcium supplementation on hip fractures and colorectal cancer. CVDs, including heart failure, were assessed as secondary outcomes. The primary eligibility for enrollment in this trial was prior enrollment in at least one of the other trials, DM and/or HT. Participants who reported use of personal vitamin D ≤ 600 IU/day and/or calcium ≤ 1,000 mg/day supplements were allowed to continue taking them even after enrolling in the CaD trial. The personal vitamin D supplement consumption was raised to an upper limit of 1,000 IU/day after the IOM published new recommendations on vitamin D intake in 1999 (154). A total of 68,132 (27,347 HT and 48,835) participants were screened for eligibility and recruitment into the CaD trial. Details of participant selection for the CaD trial and the present analyses are reported in a schematic flow diagram (Figure 4) based on CONSORT's guidelines (155).

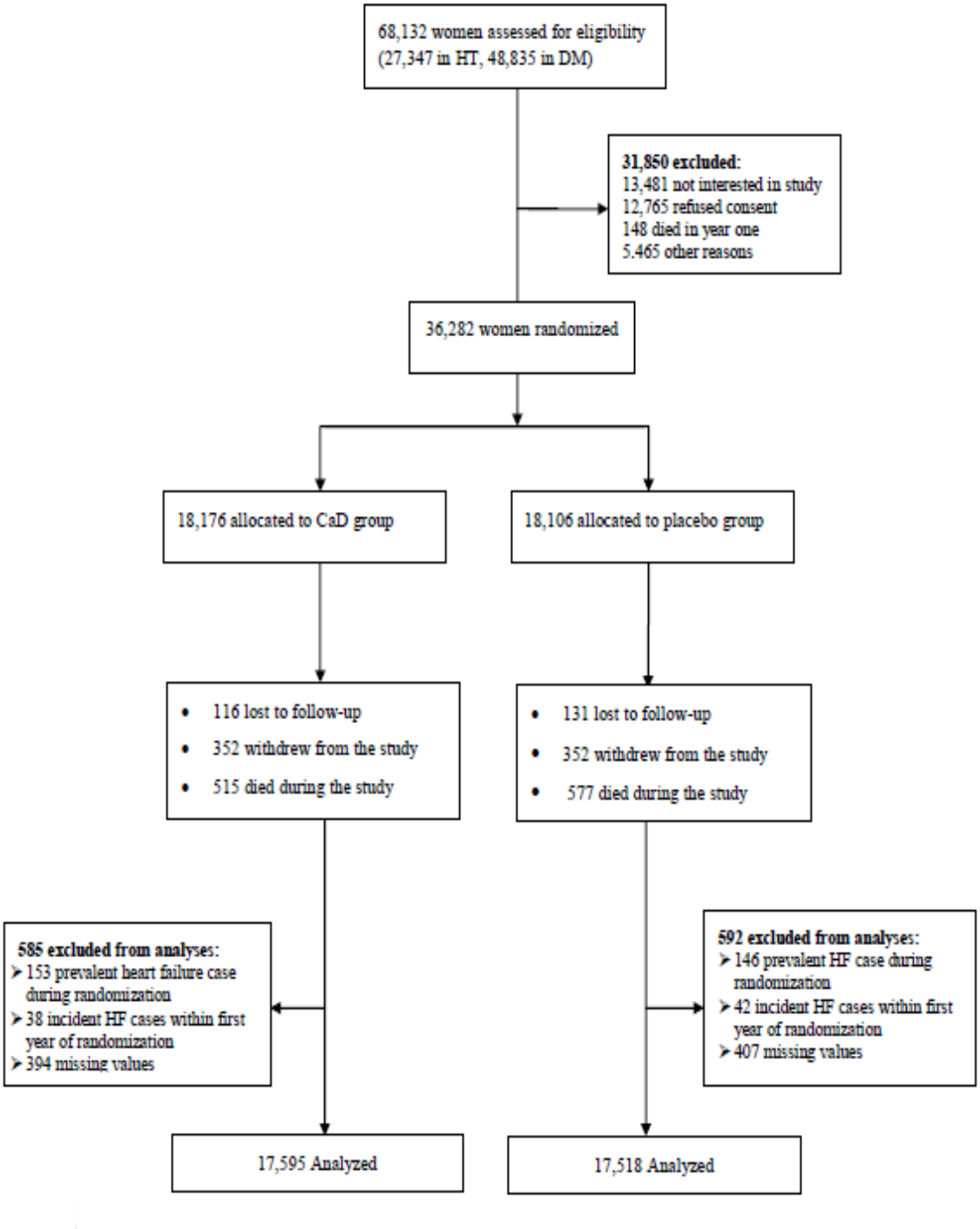


Figure 4. Participant recruitment and selection for the CaD trial and analysis

3.4.1 Eligibility Criteria

In addition to the exclusion criteria reported by Hays et al. (2003) for the three CTs (Table 1), participants were excluded from the CaD trial for safety reasons if they reported history of renal calculi, hypercalcemia, or use of corticosteroids or calcitriol (156). To reduce the effect of competing risks, individuals were excluded if they had any medical conditions associated with less than a 3 year survival. Additionally, with intention to continue consuming vitamin D supplements at dosages ≥ 600 IU/day and calcium supplements at dosages ≥ 1,000 mg/day were excluded. Based on these restrictions, 31,850 women were ineligible to participate in the CaD trial.

Women were eligible for recruitment if they were between 50 to 79 years of age and postmenopausal. In addition to providing informed consent, other eligibility criteria were based on adherence (willingness to take study pills and symptom management) and retention (intention to reside in current area for at least 3 years and attend clinic sessions). After meeting these requirements, 36,282 participants were asked to sign informed consent forms to indicate their willingness to participate in the CaD trial.

Table 1

WHI inclusion and exclusion criteria

Component	Inclusion	Exclusion
Clinical trial and observational study	50–79 years of age Postmenopausal If age <55, no menstrual period for at least 6 months If age 50–54, no menstrual period for at least 12 months Ability and willingness to provide written informed consent (component specific) Intention to reside in area for at least 3 years	
Clinical trial and observational study		Competing risk: Any medical condition with predicted survival of < 3 years Adherence or retention reasons: Alcohol or drug dependency Mental illness, including severe depression Dementia Active participation in other randomized intervention trial
Clinical trial		Competing risk: Any invasive cancer in previous 10 years Breast cancer at any time Mammogram or CBE findings suspicious of breast cancer MI in previous 6 months Stroke or TIA in past 6 months Chronic hepatitis or severe cirrhosis Safety reasons: Severe hypertension (systolic BP > 200 mm Hg or diastolic BP > 105 mm Hg) Severely underweight (BMI _18 kg/m2) Hematocrit < 32% Platelets < 75,000 cells/ml Current use of oral daily corticosteroids Adherence or retention reasons: Unwilling to participate in baseline or follow-up examination components
Dietary modification (DM)		Adherence or retention reasons: Special dietary requirements incompatible with the intervention (e.g., celiac sprue) On a diabetic or low salt diet Gastrointestinal conditions contraindicating a high fiber diet Type 1 diabetes Colorectal cancer at any time Routinely eat ≥10 meals per week prepared out of the home Unable to keep a 4-day food record

	FFQ percent calories from fat <32% FFQ energy intakes <600 or >5000 kcal Previous bilateral prophylactic mastectomy
Postmenopausal hormone therapy (PHT)	Safety reasons: Endometrial cancer at any time Endometrial hyperplasia Malignant melanoma at any time History of pulmonary embolism or deep vein thrombosis Previous osteoporosis-related fracture being treated with hormones History of bleeding disorder requiring transfusion History of hypertriglyceridemia Currently on anticoagulants Currently on tamoxifen Abnormalities in baseline pap smear, pelvic exam, or pelvic ultrasound Adherence or retention reasons: Severe menopausal symptoms that would make placebo treatment intolerable Inadequate adherence to placebo run-in Unable or unwilling to discontinue use of PHT or testosterone Refusal to have baseline endometrial aspiration
Vitamin D and calcium (caD)	Safety reasons: History of renal calculi or hypercalcemia Current use of oral corticosteroids or calcitriol Intention to continue taking ≥600 IUs of vitamin D per day

Abbreviations: BMI, body mass index; BP, blood pressure; CBE, clinical breast exam; FFQ, food frequency questionnaire; PHT, postmenopausal hormone therapy; TIA, transient ischemic attack.
Reference: Hays J, Hunt JR, Hubbell FA, et al. The Women's Health Initiative recruitment methods and results. Ann Epidemiol 2003;13(9 Suppl):S18

3.4.2 Characteristics of the CaD Trial Population

Of the total CaD trial population, only 13.8% (n = 5,017) of them were enrolled in both DM and HT trials and more than half were enrolled in DM alone (Figure 1). The mean age of the population was 62 ± 6.9 years with 45.5% between 60 – 69 years old. While efforts were made to increase minority populations participation, only 16.9% of enrolled participants belonged to one of the four minority or unknown race/ethnic groups (Table

2) (156). African Americans made up more than half (54.1%) of the minority and unknown racial populations.

Table 2

Summary of demographic characteristics of participants recruited into the CaD trial before randomization.

Baseline characteristics	N or Mean (± SD)	%
WHI clinical trial		
DM	20,193	55.7
HT	11,072	30.5
Both	5,017	13.8
Age (years)		
Mean	62.4 (± 6.9 years)	
50 – 59	13,422	37.0
60 – 69	16,520	45.5
70 – 79	6,340	17.5
Race/Ethnicity		
Non-Hispanic white	30,153	83.1
Black	3,317	9.1
Hispanic	1,507	4.2
Asian/Pacific Islander	722	2.0
American Indian	149	0.4
Unknown	434	1.2

3.4.2 The CaD Trial Randomization

The 36,282 postmenopausal women who met the inclusion criteria and agreed to participate in the CaD trial were randomized in a 1:1 ratio double-blind fashion to receive either the intervention or placebo. The intervention group was supplemented with tablets composed of calcium carbonate (containing 500 mg of elemental calcium) and 200 IU vitamin D_3. These pills were initially in chewable forms but after October 1997 swallowable forms were made available to enhance participants' tolerance to study medication (153). Participants were instructed to take these tablets twice daily with meals to maximize the absorption of vitamin D and calcium. Hence, the intervention group received a total dosage of 1,000 mg calcium and 400 IU vitamin D per day (147, 153). This dose, according to the IOM, was sufficient to meet the recommended daily allowance (RDA) of both vitamin D and calcium in postmenopausal women. Participants randomized to the placebo group were provided 'inactive' tablets which looked exactly like the 'active' tablets and instructed to take these tablets in the same format as those in the intervention group to preserve the blinding of the randomization to both study staff and participants (153).

3.4.3 Adherence to Intervention

WHI pre-defined adherence in the WHI manual as taking CaD study pills that amount to at least 80% of the appropriate amount (157). During annual and semi-annual visits, study staff weighed pills returned from participants before new ones were dispensed. Adherence was then estimated by dividing the difference between the weights of the original and returned pills by the original weight. These routine monitoring of

adherence to study medication allowed WHI to assist and encourage participants with low adherence rate to improve their adherence. One of the programs implemented by WHI to improve adherence was the "Intensive Adherence Program" through which study staff contacted low adherence participants outside the routine study visits. Details of this program and issues on adherence are found in the WHI study manual (157). After eight years of follow up, 59% of the population was observed to be taking at least 80% of their medication, Figure 5 (108).

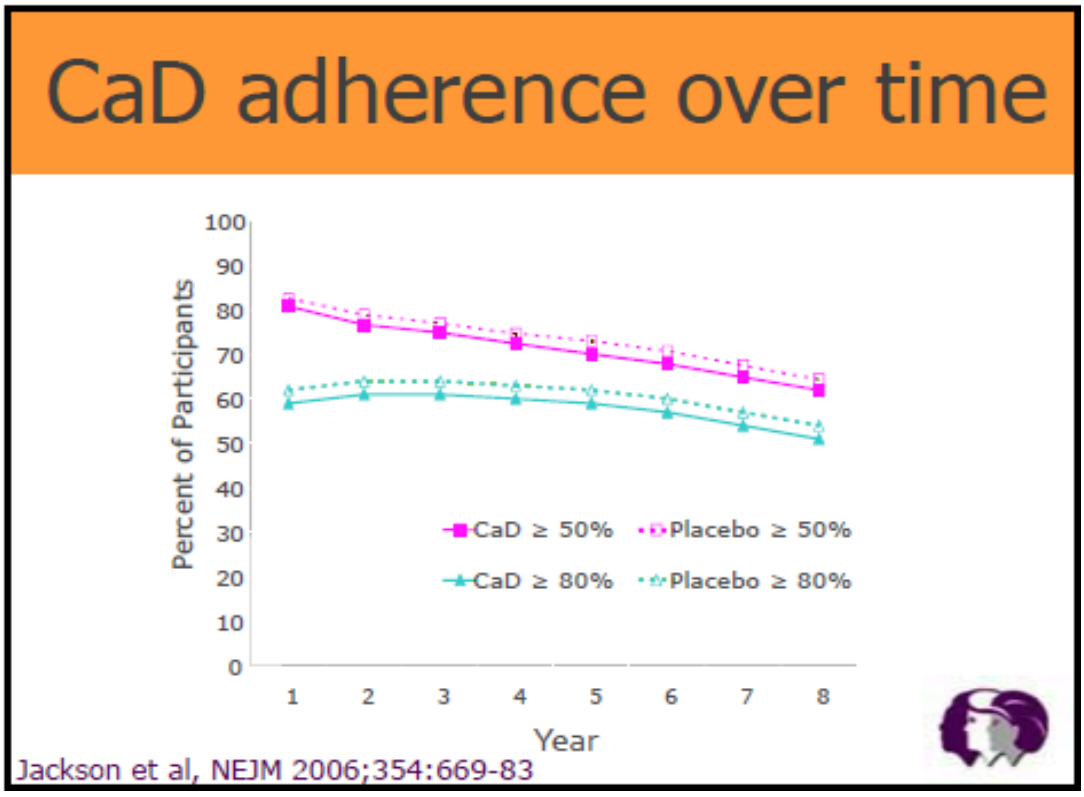


Figure 5. Adherence rate to study protocol in the vitamin D plus calcium trial of the WHI

3.4.4 Data Collection Methods

A total of 40 clinical centers were established throughout the United States of America to recruit postmenopausal women into any of the three CTs or the Observational study. At entry into the CaD trial, participants' demographic and medical history, including heart failure, were assessed through administration of standardized questionnaires. Participants were also invited to clinic centers for physical measurements on blood pressure, anthropometry, and blood samples.

CaD participants were also contacted 4 weeks into the study to complete safety and management issues on adherence, symptoms, and pill tolerance. This was then repeated semi-annually as long as participants were on study pills. Routine follow-up and data collection on CaD participants were carried out by the respective trial centers at which they were recruited.

Safety and adherence concerns were regularly addressed during routine follow-up visits. Participants were evaluated for safety concerns like renal calculi and constipation and advised on how to manage the symptoms. Trained clinic staff also followed guidelines to step-down study pills to about one pill a day when participants experienced these symptoms or had difficulties taking their pills. Participants who developed medical conditions such as hypercalcemia, renal calculi, need for hemodialysis or started taking certain medications (listed on the CaD section study manual) were taken off of the study pills without unblinding their study arm (153, 157). WHI staff recorded the dates and

specific reasons for taking participants off study medications. Details about study recruitment methods have already been published by Hays et al. (2003) (156).

3.5 Rationale for Analyzing the CaD Trial Data from the WHI Study

Even though controlled intervention studies are considered the gold standard for testing causal inference, they are lengthy and expensive to conduct. The WHI CTs, including CaD trial, are multi-million dollar well-conducted studies among postmenopausal women. Hence, the WHI data is an ideal source to perform secondary analysis of the effect of vitamin D (with calcium) supplementation on heart failure.

The WHI study is rich with a large nationally representative sample of postmenopausal women for whom quality exposure and outcome data were recorded (146). Specifically, vitamin D and calcium supplementation was randomized in a primary prevention cohort with annual assessment CVD outcomes including hospitalization for heart failure. This type of data is vital for epidemiological analysis with a focus on health outcomes among populations. Epidemiological results from such a large study are likely to be informative for public health policy initiatives.

Experienced scientists and well-trained staff from the clinical centers ensured the execution and completion of such a study with high quality. This aspect of the WHI study is not always guaranteed for many other large studies, let alone for individual or institution based studies. Hence it is befitting to take advantage of this data for testing an important hypothesis, effect of calcium plus vitamin D supplementation on heart failure, with vital clinical and public health implications.

The current secondary analysis on the WHI data may be limited by the ascertainment of heart failure (HF) cases based on hospital discharge diagnosis. As previously discussed in Chapter 1, hospital discharge diagnoses tend to overestimate HF incidence among older populations (38). However, by readjudicating these hospital discharge diagnosed HF cases, this misclassification bias is minimized (38). The WHI readjudicated all the hospital discharge diagnosed HF cases.

3.6 The WHI Study Data Access

The WHI study data are not publicly accessible. However, individual investigators are encouraged to collaborate with WHI Principal Investigators (PI) to author manuscripts for publication in peer-reviewed scientific journals. Figure 1 in the Appendix describes the steps involved with accessing the WHI study outlined on the WHI website. For the present study, JoAnn Manson, MD, DrPH, a PI at the Women's Hospital, Havard Medical School, Boston, MA was identified as a collaborator and sponsor. A proposal was developed in collaboration with my Doctoral dissertation committee members to access the WHI data. The proposal was approved by the Publications and Presentations (P&P) committee and WHI investigators nominated to join me and my dissertation committee members to constitute a writing group. The letter of approval from P&P along with the names and affiliations of the writing group can be found in the Appendix.

3.7 Assessment of Heart Failure and Other Covariates

3.7.1 Ascertainment of Heart Failure Cases

Trained staff abstracted medical records annually for self-report heart failure hospitalization. The abstracted HF cases were then classified by an adjudication committee of Physicians at local clinical sites using the criterion below:

Hospitalized HF only (HF requiring and/or occurring during hospitalization) requiring physician diagnosis of new-onset or worsened congestive heart failure on the reported hospital admission and 1 or more of the following 4 criteria:

1= HF diagnosed by physician and receiving medical treatment for HF

2= #1plus documentation in the current medical record of a history of an imaging procedure showing impaired LV systolic or diastolic LV function

3=Pulmonary edema/ congestion on chest X-ray on the current admission

4=Dilated ventricle(s) or "poor" LV or RV function by ECHO, MUGA, RVG, or other contrast ventriculography, or evidence of LV diastolic dysfunction

Description of the adjudication criterion for heart failure is found on Form 121 in the Appendix.

3.7.2 Covariates and Effect Modifiers

Stratification of study sample by preexisting diagnosed cardiovascular risk factors of heart failure

Diagnosed cardiovascular risk factors - hypertension, coronary heart disease, and diabetes - have been consistently identified by different studies in diverse populations as major risk factors for heart failure (48, 158-166). These factors are critical in the

pathogenesis of heart failure and often heart failure diagnosis. This is why the American College of Cardiology (ACC) classifies individuals with any of these risk factors with or without structural abnormalities as stage A and B heart failure. Among females in the Framingham study aged 40 -89 years, hypertension, myocardial infarction, angina, and diabetes accounted for (population attributable risk) 59%, 13%, 5%, and 12% of heart failure incidence respectively (167). However, among participants aged between 70 – 79 years old, systolic blood pressure (>140 mmHg), coronary heart disease, and fasting blood glucose (> 125 mg/dL) accounted for 21.3%, 23.9% and 11.3% respectively in the Caucasian population and 30.1%, 29.5% and 7.3% respectively in the African American population (166). In the Cardiovascular Health Study (CHS), systolic blood pressure > 140 mmHg and coronary heart disease accounted for 12.8% and 13.1% of heart failure incidence (48). These population attributable risk estimates suggest heart failure incidence is relatively higher in subpopulations with diagnosed history of hypertension, coronary heart diseases, and diabetes.

The CaD cohort was stratified into two groups; one consisted of participants who reported any history of diagnosed hypertension, cardiovascular disease, coronary heart disease/events, or diabetes, while the other group consisted of participants without any history of a previous diagnosis of any of these heart failure risk factors. Given that low serum 25(OH)D levels have been consistently associated with these same heart failure risk factors (CVD, hypertension, and diabetes), supplementation with calcium plus vitamin D may be associated with a relatively stronger effect estimates in this group compared to those without these risk factors. We hypothesized that the association between the vitamin D plus calcium supplementation and heart failure incidence is

modified by these baseline cardiovascular risk factors of heart failure. This study aims to estimate the effect of the intervention in the two groups classified based on the presence or absence of these risk factors.

However, the practice of stratified or subgroup analyses in estimating the impact of an intervention in randomized controlled trials has been debated in the scientific community (168, 169). According to Lagakos (2003), subgroup analysis, when performed and reported in a responsible manner, has great utility in clinical practice (168). Conversely, when not appropriately conducted, subgroup analysis may be misleading. To improve the translation of research results into clinical practice, Rothwell et al. suggested subgroups be identified according to existing risk models used in clinical practice (170). The cardiovascular risk factors used for grouping participants in this study are components of existing heart failure risk models from the Framingham and the Health ABC heart failure risk models (19, 171).

Description of diagnosed cardiovascular risk factors of heart failure

Hypertension: Two blood pressure (BP) measurements were taken at least 30 seconds apart during enrollment and subsequent bi-annual visits. The average of the two BP measurements at enrollment into the CaD trial was recorded and used in the definition of hypertension in these analyses. History of hypertension or high BP diagnosis by a physician was also ascertained during enrollment through questionnaires. Participants were also asked if they were taking antihypertensive medication. Based on these data, hypertension was defined as a self-report of a physician diagnosis of hypertension with or without current use of antihypertensive medications or high BP (systolic BP >140 mmHg

and/or diastolic BP >90 mmHg) during enrollment into the CaD trial. This definition was used to account for participants missing information for responses to the questions on hypertension and for those who had elevated BP but had not being diagnosed. Based on only participants' responses, WHI estimated 21,772 (66.6%) as not hypertensive, 2,644 (8.1%) as untreated hypertensive, and 8,279 (25.3%) as treated hypertensives while 3,587 were missing information for this variable. Comparatively, our definition identified 21,484 (59.2%) as never hypertensive, 4,142 (11.4%) as treated hypertensives, and 10,656 (29.4%) as treated hypertensives.

Cardiovascular diseases (CVD): During enrollment into the CaD trial, and the WHI study in general, participants were asked if a doctor has ever told them if they had heart problems, problems with blood circulation or blood clots. A positive response (n = 4,749) to this question was indicative of a previously diagnosed CVD. Missing responses (n = 3,654) were recoded as not having known CVD.

Coronary heart diseases (CHD): CHD was defined as a composite of self-reported cardiac arrest, angina, coronary artery bypass graft surgery (CABG), and/or percutaneous transluminal coronary angioplasty (PTCA). Below are the questions that were asked on the questionnaire to ascertain these conditions or procedures. A positive response to at least one of these indicted CHD.

"Please mark the conditions or procedures below that a doctor said you had";

i. Cardiac arrest – "Cardiac arrest (where your heart stopped and needed to be restarted)". A total of 28,323 (78.1%) with missing responses to this question were recoded as not having a history of cardiac arrest while only 93 responded positive.

ii. CABG – “Heart bypass operation or coronary bypass surgery for blocked or clogged arteries in your heart”. Only 232 participants provided a positive response while 436 (1.2%) participants had missing responses for this question and were recoded as never had a CABG.

iii. PTCA – “Angioplasty of the coronary arteries (opening the arteries of the heart with a balloon or other device, sometimes called a PTCA)”. Only 417 participants responded with a positive answer while 430 (1.2%) had missing responses for this question and were recoded as never had a PTCA.

iv. Angina – “Did a doctor ever say that you had angina (chest pains from a heart problem)?”. There were 1,595 positive responses and 197 (0.5%) participants with missing responses who were recoded as never had angina.

Diabetes: Diabetes was defined as a positive response to the question “Did a doctor ever say that you had sugar diabetes or high blood sugar when you were not pregnant?” during enrollment into the CaD trial and the WHI study in general. The 9 (0.02%) participants with missing information were considered nondiabetic.

3.7.3 Other Potential Covariates

Risk factors of low vitamin D status and heart failure which were measured by the CaD trial at baseline were evaluated as potential covariates for the association between the intervention and heart failure incidence.

Demographic factors: Demographic factors included age (in years), race/ethnicity (Non-Hispanic white, Black/African American, Hispanic/Latina, and others), educational level,

family income, and solar irradiance of region of residence in Langley units (172). The solar irradiance of region of residence is a measure of the amount of solar energy per unit area on the earth's surface and is an important determinant of circulatory serum vitamin D levels synthesized in the skin from UV radiations (68).

Lifestyle/behavioral factors: Lifestyle/behavioral risk factors of heart failure or low vitamin D status include smoking status, alcohol consumption and physical activity (total MET-hours/week). Self-reported personal consumption of calcium, vitamin D, and multivitamin supplements and use of medication for hormone therapy in the past two weeks and/or from prescribed medication were also included.

Physical measures: Physical measurements included as covariates were body mass index (kg/m^2), systolic and diastolic blood pressure (mmHg), and hypercholesterolemia (physician diagnosis of high cholesterol requiring medication).

Dietary factors: Dietary intake factors were measured with food frequency questionnaires (FFQ) administered during enrollment. Calcium consumption (mg/day) and vitamin D (IU/day) from dietary sources only, were calculated and included in the analyses.

3.8 Sample Selection and Power Analyses

3.8.1 Sample

In addition to the exclusion criteria applied in the CaD study, prevalent heart failure cases at enrollment and heart failure cases occurring within the first year of randomization in the CaD trial were excluded (191 intervenion, 188 control) in this analysis to create a primary prevention cohort of participants free from heart failure.

Participants missing data for pertinent variables such as BMI, systolic and diastolic blood pressures were also excluded. The details are presented in Table 3 and Figure 2. All missing data for other categorical variables were assigned a value for the worse category of the variable. These exclusion criteria reduced the study population to 35,113 (17,595 intervention and 17,518 placebo), a 3.2% reduction of the original population of 36,282.

Table 3

Frequency of missing values for select covariates by study allocation

Variables	Intervention, n (%)	Placebo, n (%)	Total
BMI	382 (49.6)	388 (50.4)	770
Systolic blood pressure	235 (49.4)	241 (50.6)	476
Diastolic blood pressure	236 (49.4)	242 (50.6)	478

3.8.2 Power Analyses

The power and sample size calculator software PS (version 3.0.43) was used to estimate the critical value of hazard ratios at the 0.05 alpha level for the two primary hypotheses given the sample sizes (173). Below are the parameters that were incorporated into the software to generate a range of detectable hazard ratios under different conditions.

a. Alpha, α – this specifies the level of type I error. A 0.05 alpha level was chosen to allow only a 5% chance of wrongly rejecting the null hypothesis for the alternate when the null is indeed true.

b. Power – this specifies the probability of correctly rejecting the null hypothesis when it is false. An 80% power was specified to estimate the hazard ratio for the association between the intervention and heart failure.

c. Sample size, N – three different sample sizes were specified. For the primary hypothesis 1a, the sample size was the entire CaD cohort, N = 35,113. While for primary hypothesis 1b, the sample sizes were specified for the two populations stratified by baseline risk for heart failure. Hence, N = 18,097 for the low risk population and N = 17,016 for the low risk population.

d. Median survival time for the placebo group – the median survival time could not be estimated from literature since there are no published data. It could not also be estimated from Kaplan-Meier curves - given that this is a retrospective power analysis after the CaD trial had completed – because more than 50% of the control group did not experience a HF incidence at the end of the 9.8 year trial period. Hence, the median survival time was estimated indirectly with equation (1).

Median survival = $\mathbf{T_0(\ln 1/2)/\ln(P)}$ (1)

where **P** = probability of survival at time = $\mathbf{T_0}$;

T_0 was specified as the total study time, 9.8 years

e. The ratio of sample size of the placebo group to the intervention group. Table 4 and Figures 6a, 6b, and 6c summarize the output from the software, PS.

Table 4

Computed critical hazard ratios for the three different populations from PS (version 3.0.43)

	Sample size (N)	Power, %	Alpha (α)	Critical hazard ratio for intervention compared to placebo (≤)
Entire CaD cohort	35,113	80	0.05	0.82
No preexisting diagnosed cardiovascular risk factors of HF	18,097	80	0.05	0.67
Preexisting diagnosed cardiovascular risk factors of HF	17,016	80	0.05	0.79

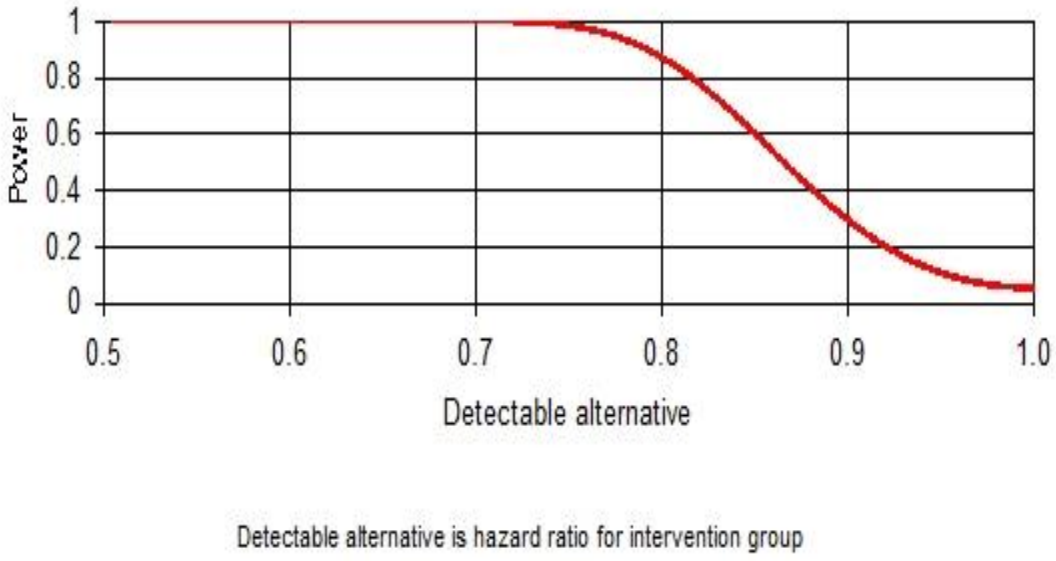


Figure 6. Plot of statistical power with detectable hazard ratios for the entire CaD trial cohort (n=35,113) analyzed.

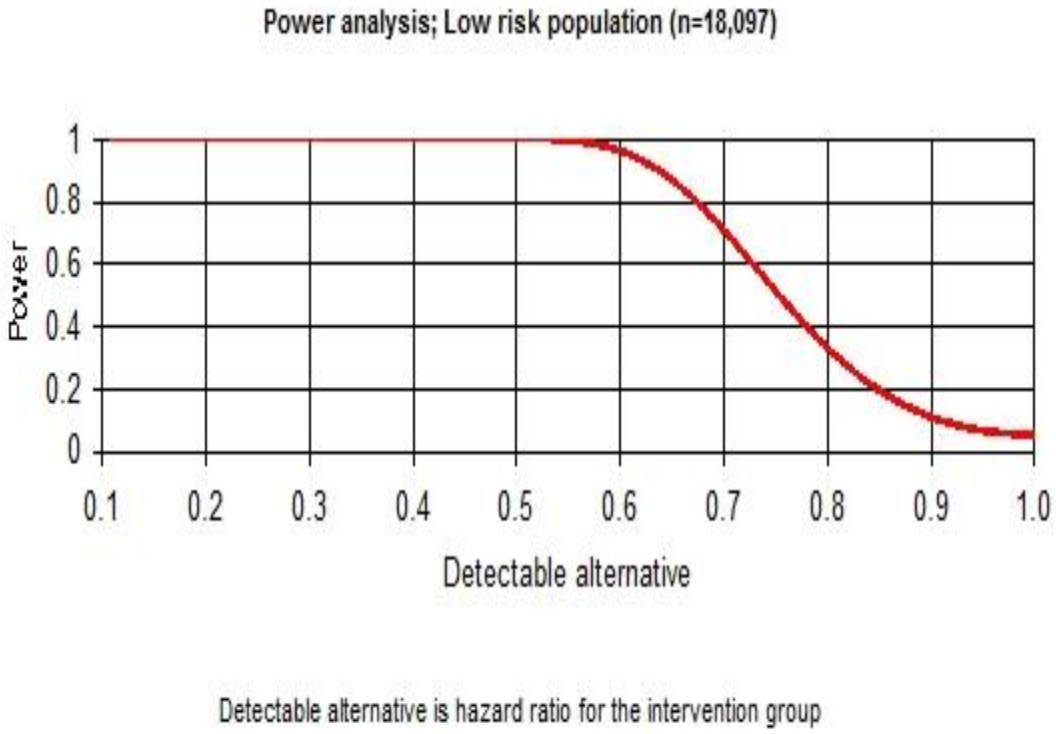


Figure 7. Plot of statistical power with detectable hazard ratios for subgroup of the CaD trial participants without any preexisting CV risk factors at baseline (n= 18,097)

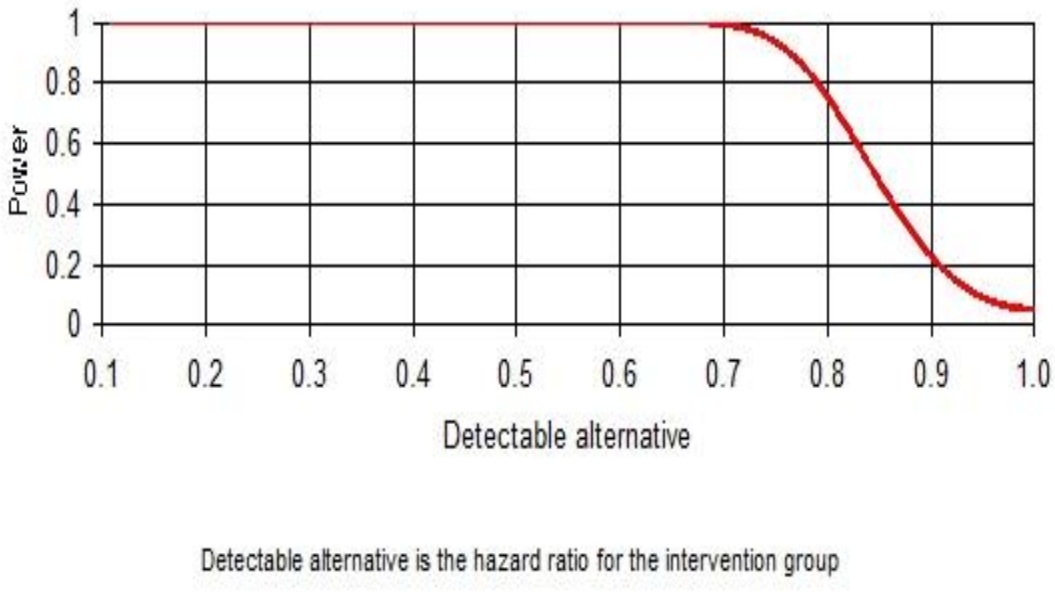


Figure 8. Plot of statistical power with detectable hazard ratios for subgroup of the CaD trial participants with at least one preexisting CV risk factor at baseline (n= 17,016).

3.9 Statistical Analysis

All statistical analyses were conducted with SAS (version 9.3). The intention-to-treat (ITT) approach was adopted for all primary analyses to evaluate the effect of CaD on the incidence of heart failure. ITT has been adopted and mandated by the Food and Drug Administration (FDA) as the preferred approach for analyzing randomized clinical trials (174). The American Statistical Association defined ITT as

"one which includes all randomized patients in the groups to which they were randomly assigned, regardless of their compliance with the entry criteria, regardless of the

treatment they actually received, and regardless of subsequent withdrawal from treatment or deviation from the protocol" (175).

The advantages of adopting the ITT approach include the ability to detect small effects because of enhanced statistical power and the ability to answer pragmatic questions (176). The ITT approach is particularly beneficial for evaluating the efficacies of treatments at the population level where not every individual prescribed a particular treatment essentially adhere to their treatment regimen. However, the ITT is not ideal when the goal of trial is to evaluate the effectiveness of a new treatment compared to a placebo given all participants adhere to the treatment (177).

3.9.1 Descriptive Statistical Analyses

Formal tests (Chi-square or Fisher exact tests) of balance of individual categorical covariates between the CaD and control arms were conducted. The Student's t-test was used to test the balance of continuous variables between the CaD and control arms. These tests were repeated for the two trial cohorts created by stratifying the CaD trial population based on the composite of baseline preexisting diagnosed cardiovascular risk factors of HF.

3.9.2 Multivariable Analyses

Adopting an intention-to-treat (ITT) approach, the effect (hazard ratio) of calcium plus vitamin D supplementation on the risk of hospitalization for heart failure was assessed through Cox Proportional Hazard regression (CPH) models for the entire cohort selected for this study. The Cox hazard regression model proposed by Sir David Cox (1972) is used to estimate the effect of a variable or intervention under the assumption that the hazard ratio, that is ratio of hazard functions between the strata of any categorical

variable, is constant over time (178). This assumption is referred to as the proportionality assumption. It is therefore important to ensure that the proportionality assumption is not violated by the covariates included in the Cox hazard regression.

Graphical methods such as the Kaplan-Meier (KM) plots of cumulative incident curves and the Schoenfeld residual plots were used to estimate the proportionality assumption in this analysis. Additionally, a non-graphical or numerical method based on the statistical significance of interaction terms between covariates and the time variable estimated from a time-dependent Cox regression model was used. By the KM approach, the assumption is not violated if the cumulative incidence curves are parallel over time or without considerable overlap over time. The KM method is quite subjective since investigators have different interpretations of what constitutes 'parallel'. Schoenfeld proposed a more objective graphical method to test the proportionality assumption by plotting scaled Schoenfeld residuals that are independent of time against time in a generalized linear regression model. If the slope of this plot is not zero, i.e. not parallel to the horizontal line specified in the plot at zero on the vertical axis, then the proportionality assumption is violated (179). In the time-dependent Cox regression model, interaction product terms between individual covariates that need to be evaluated for the proportionality assumption and time are added to the main effects of the Cox regression model and their statistical significance evaluated individually and/or together. If the interaction term with time for an individual covariate is < 0.05 (P-value) then that covariate varies with time and hence violates the proportionality assumption (178).

Association between vitamin D and calcium supplementation and heart failure incidence

Model 1a: An unadjusted CPH regression model was fitted to assess the effect of randomization status on time-to-heart failure.

Model statement:

$$h(t|X) = h(t) \exp(X\beta) \quad (2)$$

where X = randomization status (X = 1 for CaD, X = 0 for placebo)

t = time-to first heart failure event

The hazard ratio (HR) is then estimated as

$$HR_{(CaD:Placebo)} = \exp([CaD - Placebo]\ \beta) \quad (3)$$

Model 1b: Same as *Model 1a*, but adjusted for HT and DM clinical trials by stratifying the Cox CPH model on these trials. This allows the adjustment for these trials without estimating their effect on time-to-heart failure.

$$h(t|X) = h_z(t) \exp(X\beta) \quad (4)$$

where $_z$ = study arms of HT and DM clinical trials

In this stratified model the baseline hazard, $h_z(t)$, is allowed to vary for each stratum of the HT and DM clinical trials.

Additionally, the effect of the intervention on time-to-heart failure was adjusted for demographic (age, race, educational level, income, solar irradiance) and lifestyle/behavioral (BMI and physical activity, self-reported total vitamin D and calcium

intake at baseline) factors. These covariates were adjusted for because they are important confounders known to be associated with heart failure and/or vitamin D status as discussed in the literature review section of this study.

Model 1c: In building this model, baseline preexisting diagnosed cardiovascular risk factors of heart failure (hypertension, CVD, CHD/coronary events, or diabetes) were first added to *Model 1b* to evaluate their effect on the association between the intervention and heart failure. Any change in the hazard ratio estimated in *Model 1b* would be attributable to the presences of these risk factors. The rest of the covariates, family history of CVD, alcohol intake, smoking status, hypercholesterolemia, multivitamin use, and postmenopausal therapy medication use were then added to the model.

Effect modification by preexisting baseline cardiovascular risk factors of heart failure

Statistical interaction between the intervention and a dichotomous variable indicating the presence or absence of baseline preexisting diagnosed cardiovascular risk factors for heart failure was assessed by including a product term between these two factors in the CPH models described above. The *P*-value for this interaction term was evaluated for statistical significance, *P*-interaction <0.05. For the multivariable adjusted *Models 1b* and *1c*, all covariates specified above but history of CVD, CHD, hypertension and diabetes were included in the model because the baseline risk status was a composite of these cardiometabolic diseases. If the product term was statistically significant, P-value < 0.05, stratum-specific hazard ratios were reported.

Effect modification by baseline self-reported total vitamin D and calcium intakes

To evaluate the modification role of baseline total vitamin D intake on the association between the intervention and heart failure, an interaction (product) term between the two factors was added to the CPH regression models 1b (without total vitamin D intake) and 1c (without total vitamin D intake). Since participants were allowed to continue consumption of personal vitamin D supplements, baseline vitamin D supplements intake was also evaluated as a potential effect modifier.

The role of total calcium intake at baseline as an effect modifier in the association between the intervention and heart failure, the same methodological approach implemented for vitamin D described above was adopted. Personal calcium supplement intake (only) at baseline was also evaluated as a potential effect modifier.

3.10 Sensitivity Analyses

3.10.1 Per-protocol Analyses – 80% Adherence Rate

At the end of the CaD study 17,010 participants remained in the intervention arm while 16,926 of the participants in the placebo arm remained; a 3.3% vs 3.4% loses respectively. Additionally, only 57.6% (n = 20,227) of the participants adhered to at least 80% of the study medication; the placebo group had a slightly higher 80% adherence rate, 58.8% (n = 10,296) compared to the intervention group, 56.5% (n = 9,936) (Table 5). Statistical analyses were then repeated using only the population with at least 80% adherence rate to study medication. This method contrasts the intention-to-treat (ITT) analyses and is referred to as the non intention-to-treat (non-ITT) analyses (176). In this study, the per-protocol method of non-ITT analyses was adopted to test the effectiveness

of calcium plus vitamin D as an intervention against heart failure. The disadvantage of the non-ITT method is that it tends to be underpowered due to smaller sample sizes and/or non-adherence to the intervention.

Table 5

Study protocol adherence rate

Adherence rate (%)	Randomization status, n (%)		Total, n (%)
	Intervention	Control	
< 80	7,662 (43.5)	7,228 (41.3)	14,890 (42.4)
≥ 80	9,936 (56.5)	10,296 (58.8)	20,232 (57.6)

3.10.2 Estimating Hazard Ratios Independent of Noncompliance to Study Protocol by Inverse Probability of Censored Weights (IPCW) Method

The summary hazard ratios estimated in both ITT and per-protocol analyses described above were dependent on the actual censoring distribution. A total of 1,177 (3.4%) of the study population were lost to follow-up by the end of the trial (Figure 2); these participants were right-censored in the CPH model, hence, making the estimated hazard ratios dependent on the censoring. To estimate the hazard ratios with the CPH model independent of censoring information, the inverse probability of censored weights (IPCW) method suggested by Xu and O'Quigley (2000) was implemented (180, 181). In addition to participants that were lost by the end of the trial, 88% (89.7% intervention, 86.7% placebo) of the population failed to completely (100%) adhere to the study medication. The IPCW method has been proposed by Robins and Finkelstein (2000) to

estimate treatment effects in case of noncompliance to treatment protocol in randomized trials (182). The IPCW model was adjusted for some of the factors – age, education level, use of personal calcium, vitamin D or multivitamin supplements, history of heart failure risk factors, family history of CVD, and enrollment in other clinical trials - previously reported as strong predictors of adherence to study medication in the CaD trial (183).

3.10.3 Interaction between Intervention and Baseline Serum 25(OH)D Levels

Serum 25-hydroxyvitamin D (25[OH]D) measurements were collected on 2,029 participants in the CaD trial as part of a nested case-control study to evaluate whether pre-randomization serum 25(OH)D levels modified the association between the intervention and risk of fractures (108). The DiaSorin Liason chemiluminescent immunoassay system (Stillwater, Minn.) was used to measure serum 25(OH)D levels (108). Among the sample selected for this study, 1,969 participants had measured serum 25(OH)D levels, among whom were 34 heart failure cases. A nested case-control was also conducted, similar to the one designed by Jackson et. al. (2006), to evaluate whether serum 25(OH)D modified the association between intervention and heart failure (108). Owing to the very few heart failure cases with measured serum 25(OH)D measurements, a 1:4 case to control matching was performed to create a total sample of 167 (34 cases, 133 controls). Matching was done on age (<60, 60 – 69, >69 years), race, and the solar irradiance of participant's region of residence since these are strong determinants of serum 25(OH)D levels (68, 79, 80, 86, 88, 90, 91). A conditional logistic regression model was used to evaluate statistical interaction between baseline serum 25(OH)D and intervention status.

CHAPTER 4

RESULTS

4.1 Characteristics of Participants during Randomization

The characteristics of study participants are summarized for the entire population selected for our analyses and by baseline risk status for heart failure. These characteristics were compared between the intervention and control groups.

4.1.1 Entire Study Population

Table 6 summarizes the distribution of sample characteristics at baseline by randomization status. The study population consisted of 35,113 (17,595 CaD and 17,518 control) postmenopausal women with a mean age of 62.3 ± 6.9 (SD) currently residing in the United States of America. Participants allocated to the intervention and placebo arms had similar baseline socio-demographic, physical/lifestyle, and clinical factors based on comparisons of both absolute frequencies and proportions (%) or means, as shown in Table 6. The similarities between the study arms were confirmed by non-significant p-values > 0.05 alpha-level obtained from chi-square and t-tests. Although efforts were made to recruit a lot of women from minority racial/ethnic groups, the majority of participants were Caucasian in both the intervention (83.0%) and control (83.7%) groups.

Table 6

Distribution of Participants' Characteristics by Treatment Group at Baseline - The Vitamin D and calcium (CaD) Trial of the Women's Health Initiative (WHI) Study, 1995 – 2005.

Baseline characteristics	Vitamin D and calcium (N=17,595)		Control (N=17,518)	
	n or mean	% or ± SD	n or mean	% or ± SD
Age (years)				
49 - 59	6,542	37.2	6,503	37.1
60 – 69	7,997	45.5	7,978	45.5
70 – 81	3,056	17.4	3,037	17.3
Race/Ethnicity				
White	14,609	83.0	14,667	83.7
Black	1,587	9.0	1,531	8.7
Hispanic	754	4.3	687	3.9
Other	645	3.7	633	3.6
Education				
None/grade school	377	2.1	348	2.0
High school/some college	10,837	61.6	10,833	61.8
College	1,804	10.3	1,785	10.2
Beyond college	4,557	26.0	4,552	26.0
Income ($)				
<25,000	3,699	21.0	3,673	21.0
25, 000 – 50,000	7,862	44.7	7,815	44.6
50,000 – 70,000	3,339	19.0	3,318	18.9
75,000 – 100,000	1,405	8.0	1,450	8.3
>100,000	1,290	7.3	1,262	7.2
Region by Solar Irradiance				
475 – 500	3,742	21.3	3,712	21.2
400 – 430	2,917	16.6	2,910	16.6
375 – 380	1,928	11.0	1,930	11.0
350	3,796	21.6	3,773	21.5
300 – 325	5,212	29.6	5,193	29.6
High Cholesterol requiring pills ever				
No	15,679	89.1	15,643	89.3
Yes	1,916	10.9	1,875	10.7
BMI (kg/m^2)				
18.00 – 24.99	4,925	28.0	5,062	28.9
25.00 – 29.99	6,257	35.6	6,193	35.5
>30.00	6,413	36.5	6,263	35.8
Alcohol intake				
None	1,943	11.0	1,945	11.1
Past drinker	3,033	17.2	3,086	17.6
Less than 1 drink/month	2,451	13.9	2,414	13.8
Less than 7 drinks/week	8,316	47.3	8,224	47.0
More than 7 drinks/week	1,852	10.5	1,849	10.6

Physical activity level (total METs/week)				
Low	6,764	38.4	6,729	38.4
Moderate	2,271	12.9	2,292	13.1
High	8,560	48.7	8,497	48.5
Systolic blood pressure (mmHg)	125.6	17.0	125.7	17.0
Diastolic blood pressure (mmHg)	74.5	9.2	74.5	9.2
Smoking status				
None	9,240	52.5	9,320	53.2
Past	7,004	39.8	6,888	39.3
Current	1,351	7.7	1,310	7.5
Multivitamin use				
No	11,556	65.7	11,372	64.9
Yes	6,039	34.3	6,146	35.1
Daily calcium (supplements + diet), mg				
Mean (SD)	1142.0	668.8	1143.9	670.4
< 800	9,201	52.3	9,059	51.7
800 – 1,200	3,493	19.6	3,406	19.4
> 1,200	4,904	27.9	5,059	28.9
Daily vitamin D (supplements + diet), IU				
Mean (SD)	366.8	270.9	367.5	266.4
< 200	7,890	44.8	7,795	44.5
200 - 600	7,299	41.5	7,310	41.7
> 600	2,409	13.7	2,419	13.8
Ever received hormone replacement therapy				
No				
Yes	8,507	48.4	8,353	47.7
	9,088	51.7	9,164	52.3
Hypertension				
Never	10,501	59.7	10,386	59.3
Untreated	1,981	11.3	2,066	11.8
Treated	5,113	29.1	5,066	28.9
History of CVD				
No	15,461	87.9	15,308	87.4
Yes	2,134	12.1	2,210	12.6
History Diabetes				
No	16,800	95.5	16,713	95.4
Yes	796	4.5	805	4.6
History of CHD				
No	16,135	91.8	16,120	92.1
Yes	1,441	8.2	1,383	7.9
Family history of CVD				
No	6,053	34.4	6,043	34.5
Yes	11,542	65.6	11,475	65.5
Dietary modification trial assignment				
Not randomized	5,435	30.9	5,320	30.4
Intervention	4,622	26.3	4,729	27.0
Control	7,538	42.8	7,469	42.6
Hormone therapy trial assignment				
Not randomized	9,762	55.5	9,722	55.5
Estrogen alone intervention	1,477	8.4	1,483	8.5
Estrogen alone control	1,483	8.4	1,519	8.7
Estrogen + progesterone intervention	2,445	13.9	2,467	14.1
Estrogen + progesterone control	2,428	13.3	2,327	13.3

Abbreviations: SD, standard deviation; BMI, body mass index; MET, metabolic equivalent of task; CVD, cardiovascular disease; CHD, coronary heart disease.

4.1.2 Population stratified by baseline risk status for heart failure

The secondary hypothesis of this study was to evaluate and compare the effect of CaD on baseline risk status for heart failure. The distribution of participant characteristics by intervention status at baseline was evaluated for the high and low risk populations. Per our definition, 17,016 participants (8,494 CaD and 8,522 placebo) reported at least one preexisting diagnosed cardiovascular risk factor of heart failure (cardiovascular diseases, coronary heart diseases, hypertension, or diabetes), while 18,079 participants (9,101 CaD and 8,996 placebo) were free of these heart failure risk factors at baseline. The baseline characteristics of participants free of preexisting diagnosed cardiovascular risk factors are presented in Table 7. As observed in the sample selected for this analysis, the baseline participants' characteristics were balanced between the intervention and placebo groups by randomization (Table 7) for this group. Similarly, all baseline characteristics of the group with preexisting diagnosed cardiovascular risk factors of heart failure were proportionally distributed between the intervention and control groups with the exception of body mass index (BMI) (Table 8). A chi-squared p-value of 0.04 was observed for the test of independence between the intervention and placebo groups by three categories of BMI (18.00 – 24.99, 25.00 – 29.99, and >30.00). However, there was no significant difference in the mean BMI between the intervention and control groups, 30.03 vs 30.06 kg/m^2, $P = 0.71$ (Table 8).

Table 7

Baseline Characteristics of Participants Without Preexisting Cardiovascular Risk Factors of Heart Failure (N = 18,097) by Treatment Group - the Vitamin D and calcium (CaD) Trial of thhe Women's Health Initiative (WHI) Study, 1995 – 2005.

Baseline characteristics	Vitamin D and calcium (N = 9,101)		Control (N = 8,996)	
	n or mean	% or ± SD	n or mean	% or ± SD
Age (years)				
49 - 59	4,020	44.2	3,975	44.2
60 – 69	3,916	43.0	3,883	43.2
70 – 81	1,165	12.8	1,138	12.7
Race/Ethnicity				
White	7,832	86.1	7,824	87.0
Black	534	5.9	500	5.6
Hispanic	435	4.8	384	4.3
Other	300	3.3	288	3.2
Education				
None/grade school	171	1.9	148	1.7
High school/some college	5,317	58.4	5,248	58.3
College	1,009	11.1	1,004	11.2
Beyond college	2,604	28.6	2,596	28.9
Income ($)				
<25,000	1,640	18.0	1,586	17.6
25, 000 – 50,000	3,880	42.6	3,938	43.8
50,000 – 70,000	1,863	20.5	1,818	20.2
75,000 – 100,000	884	9.7	861	9.6
>100,000	834	9.2	793	8.8
Region by Solar Irradiance				
475 – 500	1,986	21.8	1,940	21.6
400 – 430	1,554	17.1	1,508	16.8
375 – 380	945	10.4	968	10.8
350	1,934	21.3	1,888	21.0
300 – 325	2,682	29.5	2,692	29.9
High Cholesterol requiring pills ever				
No	8,472	93.1	8,416	93.6
Yes	629	6.9	580	6.5
BMI (kg/m^2)				
18.00 – 24.99	3,155	34.7	3,265	36.3
25.00 – 29.99	3,355	36.9	3,301	36.7
>30.00	2,591	28.5	2,430	37.0
Alcohol intake				
None	915	10.1	900	10.0
Past drinker	1,315	14.5	1,313	14.6
Less than 1 drink/month	1,215	13.4	1,219	13.6
Less than 7 drinks/week	4,627	50.8	4,521	50.3
More than 7 drinks/week	1,029	11.3	1,043	11.6
Physical activity level (total METs/week)				

Low	3,390	37.3	3,323	36.9
Moderate	1,103	12.2	1,152	12.8
High	4,608	50.6	4,521	50.3
Systolic blood pressure (mmHg)	117.6	11.9	117.5	11.9
Diastolic blood pressure (mmHg)	72.0	7.7	71.8	7.7
Smoking status				
None	4,732	52.0	4,759	52.9
Past	3,162	39.7	3,525	39.2
Current	754	8.3	712	7.9
Multivitamin use				
No	6,000	65.9	5,821	64.7
Yes	3,101	34.1	3,175	35.3
Daily calcium (supplements + diet), mg				
Mean (SD)	1170.0	680.0	1166.5	660.3
< 800	4,664	51.2	4,526	50.3
800 – 1,200	1,805	19.8	1,748	19.4
> 1,200	2,634	28.9	2,725	30.3
Daily vitamin D (supplements + diet), IU				
Mean (SD)	368.1	273.4	368.4	267.6
< 200	4,098	45.0	3,967	44.1
200 - 600	3,760	41.3	3,793	42.2
> 600	1,245	13.7	1,239	13.8
Ever received hormone replacement therapy				
No	4,336	47.6	4,175	46.4
Yes	4,765	52.4	4,821	53.6
Family history of CVD				
No	3,506	38.5	3,446	38.3
Yes	5,595	61.5	5,550	61.7
Dietary modification trial assignment				
Not randomized	2,887	31.7	2,756	30.6
Intervention	2,381	26.2	2,447	27.2
Control	3,833	42.1	3,793	42.2
Hormone therapy trial assignment				
Not randomized	4,898	54.8	5,042	56.1
Estrogen alone intervention	638	7.0	648	7.2
Estrogen alone control	689	7.6	675	7.5
Estrogen + progesterone intervention	1,413	15.5	1,333	14.8
Estrogen + progesterone control	1,372	15.1	1,298	14.4

Abbreviations: SD, standard deviation; BMI, body mass index; MET, metabolic equivalent of task; CVD, cardiovascular disease.

Table 8

Baseline Characteristics of Participants with Preexisting Cardiovascular Risk Factors of Heart Failure (N = 17,016) By Treatment Group - The Vitamin D and calcium (CaD) Trial of the Women's Health Initiative (WHI) Study, 1995 – 2005.

Baseline characteristics	Vitamin D and calcium (N=8,494)		Control (N=8,522)	
	n or mean	% or ± SD	n or mean	% or ± SD
Age (years)				
49 - 59	2,522	29.7	2,528	29.7
60 – 69	4,081	48.1	4,095	48.1
70 – 81	1,891	22.3	1,899	22.3
Race/Ethnicity				
White	6,777	80.0	6,843	80.3
Black	1,053	12.4	1,031	12.1
Hispanic	319	3.8	303	3.6
Other	345	4.1	345	4.1
Education				
None/grade school	206	2.4	200	2.4
High school/some college	5,520	65.0	5,585	65.5
College	795	9.4	781	9.2
Beyond college	1,973	23.2	1,956	23.0
Income ($)				
<25,000	2,059	24.2	2,087	24.5
25, 000 – 50,000	3,982	46.9	3,877	45.5
50,000 – 70,000	1,476	17.4	1,500	17.6
75,000 – 100,000	521	6.1	589	6.9
>100,000	456	5.4	469	5.5
Region by Solar Irradiance				
475 – 500	1,756	20.7	1,772	20.8
400 – 430	1,363	16.1	1,402	16.5
375 – 380	983	11.6	962	11.3
350	1,862	21.9	1,885	22.1
300 – 325	2,530	29.8	2,501	29.4
High Cholesterol requiring pills ever				
No	7,207	84.9	7,227	84.8
Yes	1,287	15.1	1,295	15.2
BMI (kg/m^2)				
Mean	30.07	6.14	30.03	6.20
18.00 – 24.99	1,770	20.8	1,797	21.1
25.00 – 29.99	2,902	34.2	2,892	33.9
>30.00	3,822	45.0	3,833	45.0
Alcohol intake				
None	1,028	12.1	1,045	12.3
Past drinker	1,718	20.2	1,773	20.8
Less than 1 drink/month	1,236	14.6	1,195	14.0
Less than 7 drinks/week	3,689	43.4	3,703	43.5
More than 7 drinks/week	823	9.7	806	9.5

Physical activity level (total METs/week)				
Low	3,374	39.7	3,406	40.0
Moderate	1,168	13.8	1,140	13.4
High	3,952	46.5	3,976	46.7
Systolic blood pressure (mmHg)	134.0	17.4	134.1	17.4
Diastolic blood pressure (mmHg)	77.3	9.8	77.2	9.8
Smoking status				
None	4,508	53.1	4,561	53.5
Past	3,389	39.9	3,363	39.5
Current	597	7.0	598	7.0
Multivitamin use				
No	5,556	65.4	5,551	65.1
Yes	2,938	34.6	2,971	34.9
Daily calcium (supplements + diet), mg				
Mean (SD)	1112.7	655.8	1120.3	680.1
< 800	4,537	53.4	4,533	53.2
800 – 1,200	1,688	19.9	1,658	19.5
> 1,200	2,270	26.7	2,334	27.4
Daily vitamin D (supplements + diet), IU				
Mean (SD)	365.4	268.3	366.6	265.1
< 200	3,792	44.6	3,828	44.9
200 - 600	3,539	41.7	3,517	41.3
> 600	1,164	13.7	1,180	13.8
Ever received hormone replacement therapy				
No	4,171	49.1	4,178	49.0
Yes	4,323	50.9	4,343	51.0
Family history of CVD				
No	2,547	30.0	2,597	30.5
Yes	5,947	70.0	5,925	69.5
Dietary modification trial assignment				
Not randomized	2,548	30.0	2,564	30.1
Intervention	2,241	26.4	2,282	26.8
Control	3,705	43.6	3,676	43.1
Hormone therapy trial assignment				
Not randomized	4,773	56.2	4,680	54.9
Estrogen alone intervention	839	9.9	835	9.8
Estrogen alone control	794	9.4	844	9.9
Estrogen + progesterone intervention	1,032	12.2	1,134	13.3
Estrogen + progesterone control	1,056	12.4	1,029	12.1

Abbreviations: SD, standard deviation; BMI, body mass index; MET, metabolic equivalent of task; CVD, cardiovascular disease.

4.2 Estimation of Risk of Heart Failure (Hazard Ratios)

4.2.1. The Cox Proportional Hazard Regression Model and Test of the Proportionality Assumption

The Kaplan-Meier curves for the intervention and control groups in Figure 7 appear to be parallel although they converge at two points before diverging after approximately 6.5 years after randomization. It is therefore not clear whether the proportionality assumption was violated or not. Hence, this proportionality assumption was further evaluated with plots of Schoenfeld residuals obtained from a Cox regression model containing all covariates (179). The Schoenfeld residual plots for the intervention status (Figure 8) and the other variables confirmed that the proportionality assumption was not violated because all the plotted curves were close to zero. A time-dependent Cox regression model was also built to confirm that the proportionality assumption was met by all variables. The major predictor, intervention status, and all other covariates neither interacted with time individually or collectively, *P*-test of proportionality = 0.66. It was assumed the proportionality assumption is not violated by the randomization status based on results from both a graphical (Schoenfeld residual plots) and numerical (time-dependent Cox regression) assessment methods.

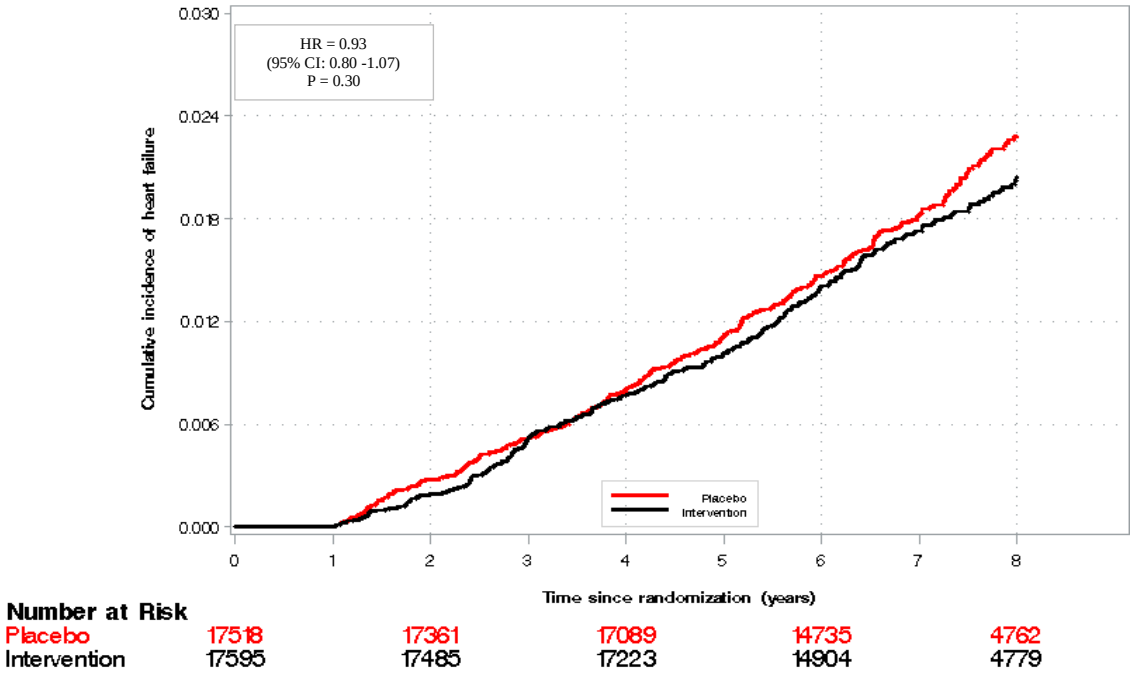


Figure 9. KM curves comparing the cumulative incidence of HF between the intervention and placebo arms during follow-up period for the overall CaD cohort.

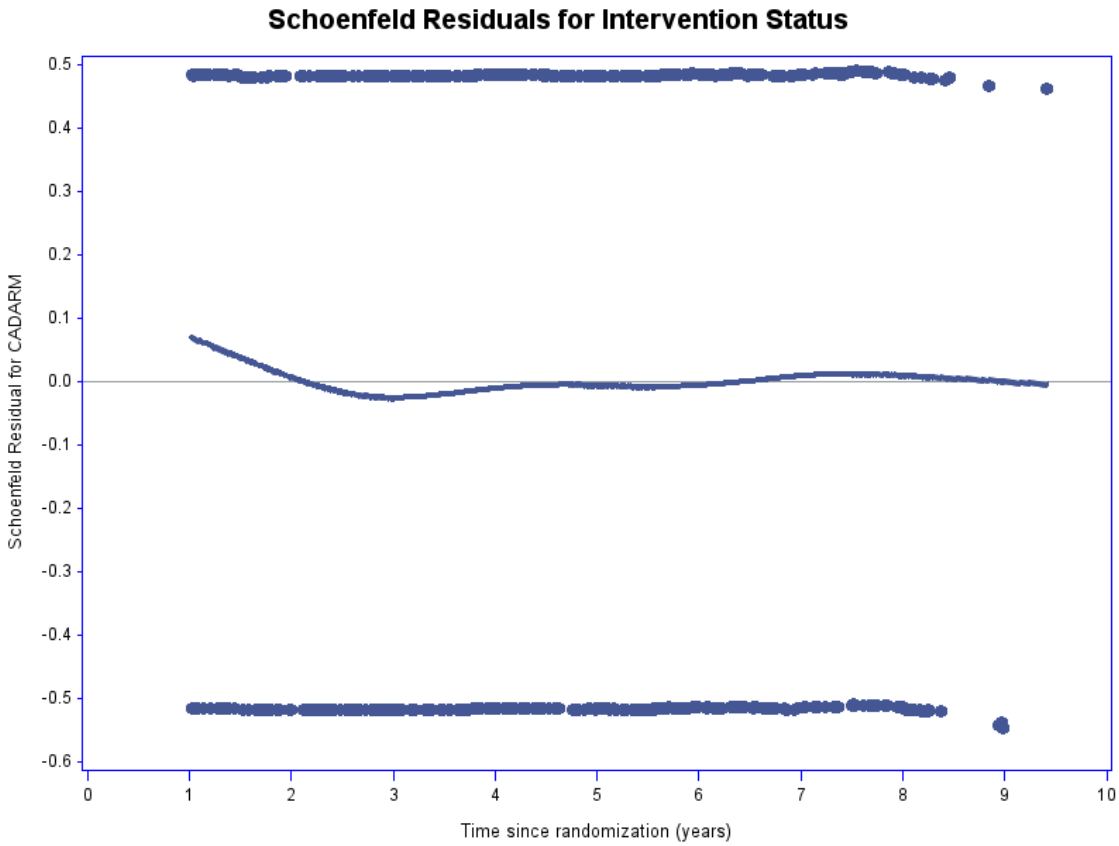


Figure 10. Schoenfeld residual curve to evaluate the proportional hazards assumption between the intervention and placebo in the overall CaD cohort.

The proportionality assumption was also evaluated using Kaplan-Meier curves, Schoenfeld residual plots, and time-dependent Cox regression model for populations stratified on the presence or absence of baseline cardiovascular risk factors of heart failure. Based on these methods, all variables, including the randomization status met the proportionality assumption (*P*-test of proportionality = 0.95) for the population without baseline cardiovascular risk factors of heart failure. Figures 9 and 10 are the Kaplan-Meier and Schoenfeld residual curves, respectively, testing the proportionality assumption for the randomization status in this population. Even though the two

cumulative incidence curves never converged or crossed, they begun to stay diverged after approximately four years after randomization (Figure 8).

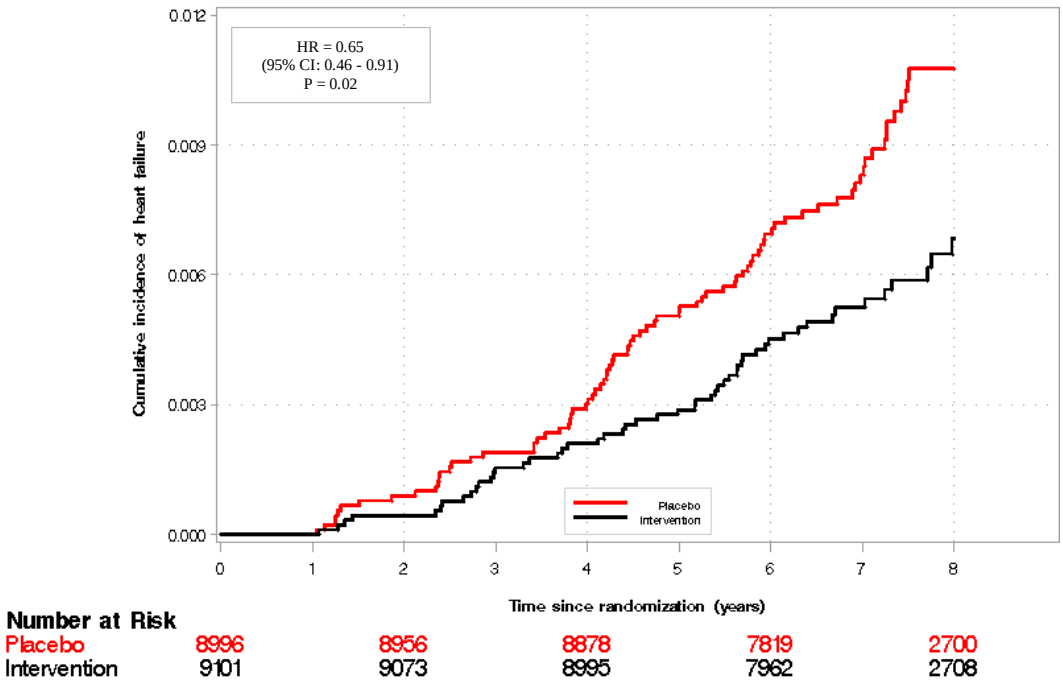


Figure 11. KM curves comparing the cumulative incidence of HF between the intervention and placebo arms during follow-up period for the subgroup of participants without any preexisting CV risk factors at baseline.

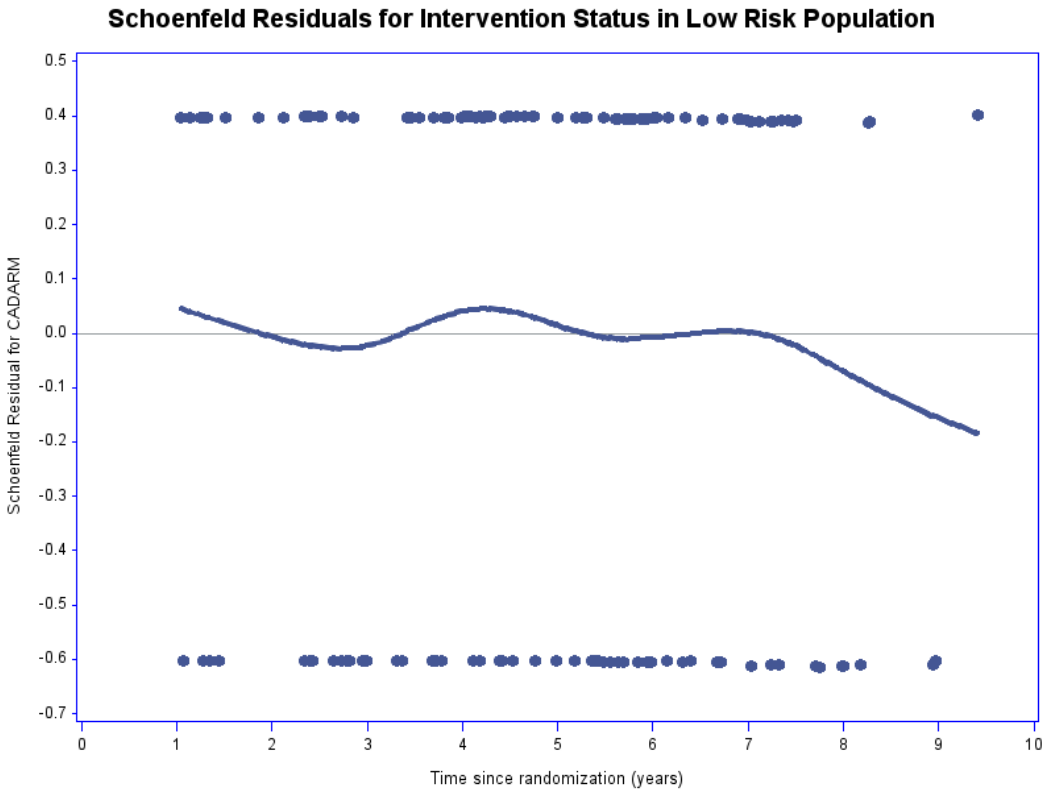


Figure 12. Schoenfeld residual curve to evaluate the proportional hazards assumption between the intervention and placebo in the subgroup of participants without any preexisting CV risk factors at baseline.

However, in the population without preexisting cardiovascular risk factors of heart failure at baseline, the cumulative incidence curves arms slightly crossed intermittently at three points (Figure 11). Further evaluations by a Schoenfeld residual plot shows a curve that is parallel to zero (Figure 12). Additionally, a time-dependent Cox regression model also indicated that none of the covariates including the randomization status interacted significantly with time (*P*-test of proportionality = 0.24). Hence, the Cox proportional hazards model was used to estimate the hazard ratio associated with CaD in this group.

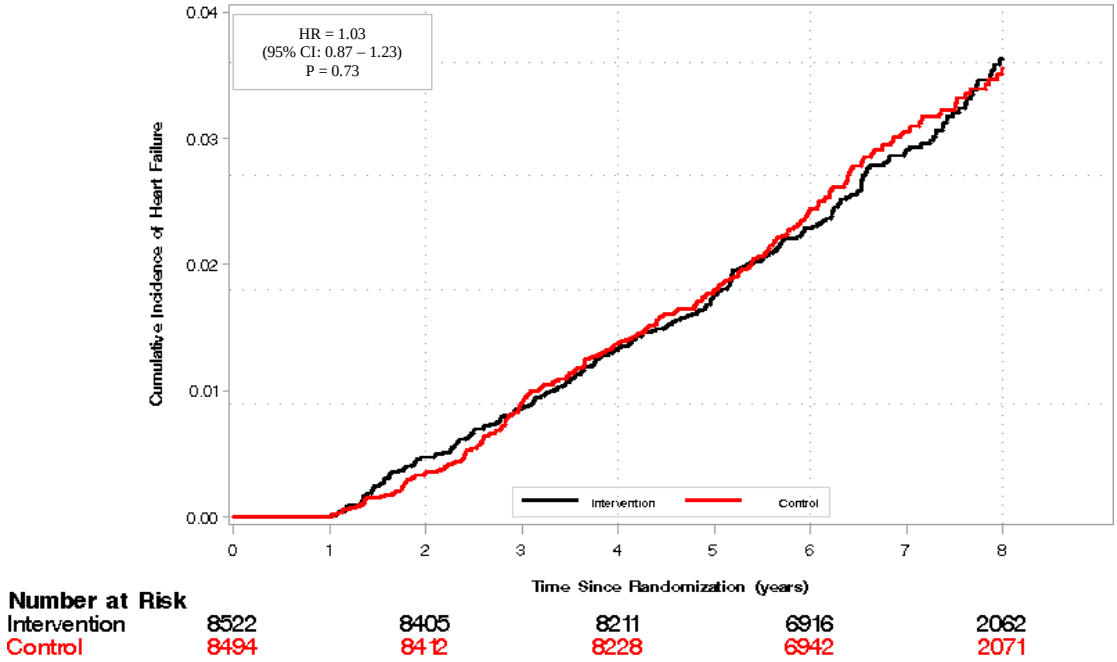


Figure 13. KM curves comparing the cumulative incidence of HF between the intervention and placebo arms during follow-up period for the subgroup of participants with at least one preexisting CV risk factor at baseline.

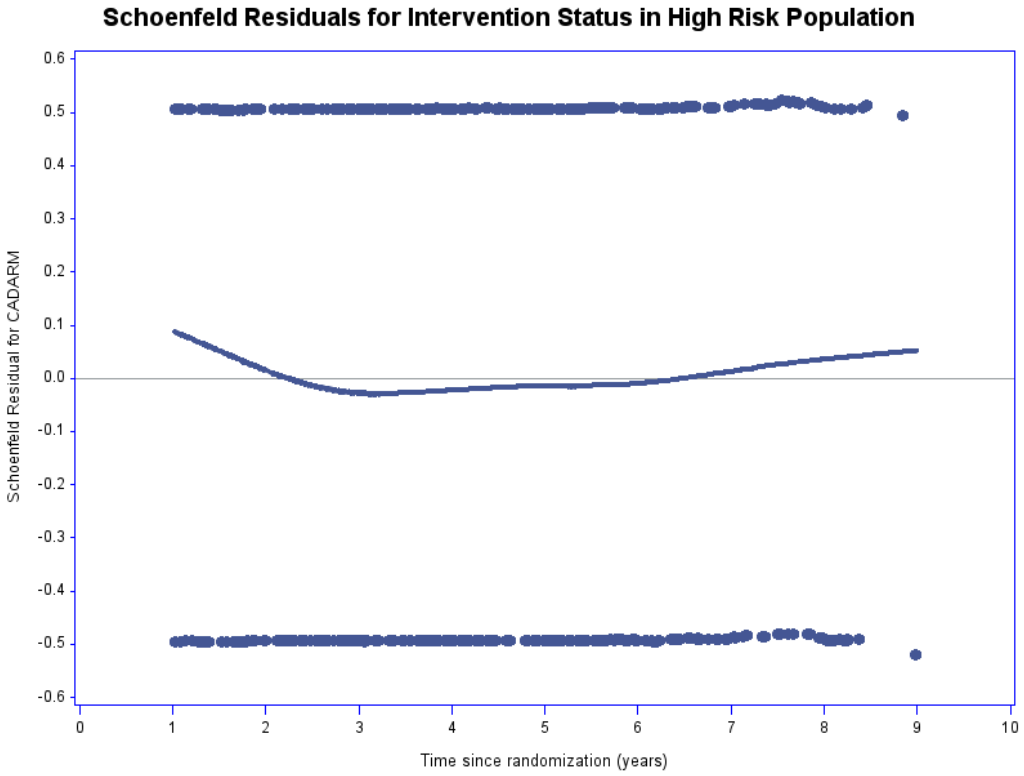


Figure 14. Schoenfeld residual curve to evaluate the proportional hazards assumption between the intervention and placebo in the subgroup of participants with at least one preexisting CV risk factor at baseline.

4.2.2 Association between Calcium (1,000 Mg/Day) and Vitamin D (400 IU/Day) Supplementation and Incidence of Heart Failure

Supplementation with vitamin D and calcium was associated with a non-statistically significant 7.0% (HR, 0.93; 95% CI, 0.80 – 1.07, *P* = 0.30) reduction in the risk of heart failure after approximately 10 years of follow-up (mean, 7.13 [SD, 1.33] years) (Table 9). When the cumulative incidence curves for the intervention and placebo groups in Figure 6 were compared by the Log-Rank test, the two curves were not significantly different, *P* = 0.36. The risk only decreased slightly after adjusting

minimally and fully for socio-demographic, physical/lifestyle, and comorbidity factors (Table 9). Again, none of these hazard ratios were statistically significant.

Table 9

Association between calcium (1000 mg/day) and vitamin D (400 IU/day) supplementation and incidence of heart failure, WHI study, 1995 – 2005.

Study arm	Heart failure cases		Hazard ratio (95% CI)		
	N	Rate *	Unadjusted	Minimally adjusted†	Fully adjusted††
Placebo (n = 17,518)	339	27.1	Referent	Referent	Referent
Intervention (n= 17,595)	318	25.2	0.93(0.80 – 1.07)	0.92(0.80 – 1.07)	0.92 (0.79 –1.06)
Age (years)					
49 - 59				1	1
60 – 69				2.73(2.16 – 3.45)	2.08 (1.63 – 2.64)
70 – 81				6.10(4.77 – 7.80)	3.58 (2.75 – 4.66)
Race/Ethnicity					
White				1	1
Black				1.16(0.90 – 1.50)	0.91(0.70 – 1.19)
Hispanic				0.89(0.58 – 1.39)	0.97(0.62 – 1.51)
Other				0.76(0.46 – 1.25)	0.61(0.36 – 1.03)
Education					
None/grade school				1	1
High school/college				0.78(0.51 – 1.22)	0.81(0.52 – 1.26)
College				0.64(0.38 – 1.08)	0.71(0.42 – 1.21)
Beyond college				0.75(0.47 – 1.20)	0.81(0.51 – 1.31)
Income ($)					
<25,000					1
25, 000 – 50,000				0.70(0.59 – 0.84)	0.76(0.64 – 0.91)
50,000 – 70,000				0.52(0.40 – 0.68)	0.59(0.45 – 0.78)
75,000 – 100,000				0.23(0.13 – 0.41)	0.29(0.16 – 0.52)
>100,000				0.49(0.31 – 0.79)	0.62(0.39 – 1.04)
Region by Solar Irradiance					
475 – 500				1	1
400 – 430				0.75(0.57 – 0.97)	0.68 (0.52 – 0.90)
375 – 380				1.13(0.86 – 1.50)	1.03 (0.78 – 1.36)
350				1.01(0.80 – 1.29)	0.95 (0.75 – 1.20)
300 – 325				0.75(0.60 – 0.95)	0.70 (0.55 – 0.88)
Physical activity level (total METs/week)					
Low				1	1
Moderate				0.85(0.67 – 1.08)	0.82(0.64 – 1.04)
High				0.72(0.61 – 0.86)	0.73(0.61 – 0.86)
BMI (kg/m^2)					
18.00 – 24.99				1	1
25.00 – 29.99				1.07(0.86 – 1.18)	0.97(0.78 – 1.22)
>30.00				1.86(1.51 – 2.29)	1.37 (1.10 – 1.70)
Daily calcium (supplements + diet), mg					
< 600				1	1
600 - 800				1.03(0.75 – 1.41)	1.06(0.77 – 1.45)
800 – 1,200				1.09(0.81 – 1.45)	1.08(0.81 – 1.45)
> 1,200				1.10(0.81 – 1.49)	1.14(0.84 – 1.53)
Daily vitamin D (supplements + diet), IU					
< 200				1	1

200 - 600	1.07(0.86 – 1.34)	1.00(0.77 – 1.29)
> 600	1.86(1.51 – 2.28)	1.06(0.74 – 1.52)
Ever received hormone replacement therapy		
No	1	1
Yes	0.87(0.74 – 1.02)	0.86(0.73 – 1.01)
High Cholesterol requiring pills ever		
No		1
Yes		1.03 (0.84 – 1.28)
Alcohol intake		
None		1
Past drinker		1.13 (0.86 – 1.47)
Less than 1 drink/month		0.93 (0.68 – 1.26)
Less than 7 drinks/week		0.99 (0.76 – 1.28)
More than 7 drinks/week		0.74 (0.50 – 1.09)
Systolic blood pressure (mmHg)		1.02(1.06 – 1.03)
Diastolic blood pressure (mmHg)		0.98(0.97 – 0.99)
Smoking status		
None		1
Past		1.23(1.04 – 1.45)
Current		1.58(1.17 – 2.12)
Multivitamin use		
No		1
Yes		0.98(0.81 – 1.18)
Family history of CVD		
No		1
Yes		1.31(1.10 – 1.57)

*Rate/10,000 person years

† stratified on clinical trial (HT/DM) and adjusted for: age, race, educational level, income, solar irradiance, BMI, physical activity, systolic blood pressure, diastolic blood pressure, calcium supplements intake, vitamin d supplement intake, use of hormone replacement therapy

†† stratified on clinical trial (HT/DM) and adjusted for all variables in † in addition to cholesterol levels, smoking status, alcohol intake, multivitamin use, family history of CVD, history of CVD, history of CHD, history of hypertension, history of diabetes

4.2.3 Association between Calcium (1,000 Mg/Day) and Vitamin D (400 IU/Day) Supplementation and Incidence of Heart Failure by Baseline Preexisting Diagnosed Cardiovascular Risk Factors of HF

Effect modification by baseline preexisting diagnosed cardiovascular risk factors of HF was evaluated by testing the statistical significance of a product (interaction) term between the intervention and an indicator variable for the composite of these HF risk

factors in both unadjusted and fully-adjusted Cox regression models. The interaction term was statistically significant in both unadjusted (*P*-interaction = 0.02) and fully-adjusted models (*P*-interaction = 0.02) indicating preexisting diagnosed cardiovascular risk factors of HF modified the association between CaD and incidence of heart failure. A Log-Rank test of the differences between the cumulative incidence rates of the intervention and control group (Figure 9) was statistically significant (*P* = 0.01).

However, individually none of the baseline cardiovascular risk factors of heart failure interacted significantly with the intervention - hypertension (*P*-interaction = 0.44), CVD (*P*-interaction = 0.24), CHD/coronary events (*P*-interaction = 0.62), and diabetes (*P*-interaction = 0.52). It is critical to note that there is considerable overlap of participants with respect to these risk factors (Tables 10 and 11). Thus, given the relatively small proportions of participants in each stratum (risk factor) and fewer heart failure events, it makes statistical sense that significant interaction was not detected at an alpha level < 0.05. It is also important not to misconstrue the lack of (partial) interaction by the individual HF risk factors, even though collectively they interact significantly with the intervention status, as a scenario of the Yule-Simpson paradox (184). A necessary condition for the Yule-Simpson paradox to occur in a 2x2x2 contingency table is that all three variables be dependent of each other (185). However, while these individual HF risk factors are associated with their composite, they are not associated with the randomization status because of the randomized study design nature (Table 6). Also, their composite was not associated with the randomization status, χ^2 (0.49), *P* = 0.49. Hence it is not likely the Yule-Simpson paradox was present in this data structure.

Table 10

Overlap of baseline cardiovascular risk factors of heart failure

Baseline cardiovascular risk factors of heart failure				Frequency	%
Hypertension	CVD	CHD	Diabetes		
No	No	No	No	18097	51.54
No	No	No	Yes	527	1.5
No	No	Yes	No	189	0.54
No	No	Yes	Yes	12	0.03
No	Yes	No	No	1616	4.6
No	Yes	No	Yes	65	0.19
No	Yes	Yes	No	346	0.99
No	Yes	Yes	Yes	35	0.1
Yes	No	No	No	10625	30.26
Yes	No	No	Yes	987	2.81
Yes	No	Yes	No	282	0.8
Yes	No	Yes	Yes	50	0.14
Yes	Yes	No	No	1438	4.1
Yes	Yes	No	Yes	158	0.45
Yes	Yes	Yes	No	554	1.58
Yes	Yes	Yes	Yes	132	0.38

Abbreviations: CVD, cardiovascular disease; CHD, coronary heart disease

Table 11

Distribution of participants by minimum number of cardiovascular (CV) risk factors of heart failure

Minimum number of CV risk factors	N	HF cases, N (%)	Randomization status (%)		HR (95% CI)
			Intervention	Control	
None	18,106	140 (0.77)	51.7	51.4	0.66 (0.47 – 0.92)
One	12,957	263 (2.03)	37.0	36.8	1.07 (0.84 – 1.37)
Two	3,130	169 (5.40)	8.7	9.1	1.06 (0.79 – 1.43)
Three	797	70 (8.78)	2.2	2.3	0.82 (0.51 – 1.31)
Four	132	16 (12.12)	0.4	0.4	1.41 (0.52 – 3.77)

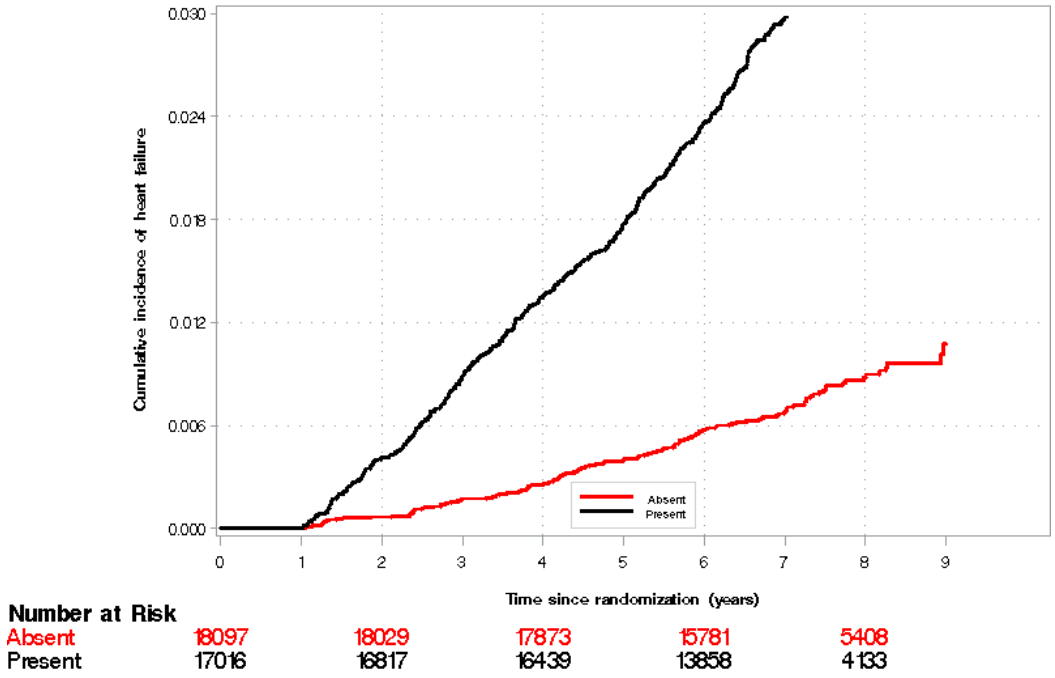


Figure 15. KM curves comparing the cumulative incidence of heart failure between populations stratified by the presence or absence of baseline cardiovascular risk factors of heart failure.

Table 12 and Figure 14 report the effect of vitamin D and calcium on heart failure stratified by individual baseline diagnosed cardiovascular risk factors of heart failure and the composite of these factors. None of these individual baseline risk factors significantly modified the effect of CaD on heart failure. However, when the hazard ratio for the intervention was stratified by the composite of these cardiovascular risk factors, the intervention was associated with a 35% reduced risk of HF, HR, 0.65; 95% CI, 0.46 – 0.87; $P = 0.01$, in the sample without preexisting cardiovascular risk factors of HF at baseline. This association persisted after adjusting for a host of risk factors of heart

failure and low vitamin D status, HR, 0.65; 95% CI, 0.46 – 0.92; *P* = 0.01. However, in the sample with preexisting baseline cardiovascular risk factors, CaD did not affect risk of HF in both unadjusted (HR, 1.03; 95% CI, 0.87 – 1.23; P = 0.73) and multivariable-adjusted (HR, 1.02; 95% CI, 0.85 – 1.21; P = 0.87) models.

Table 12

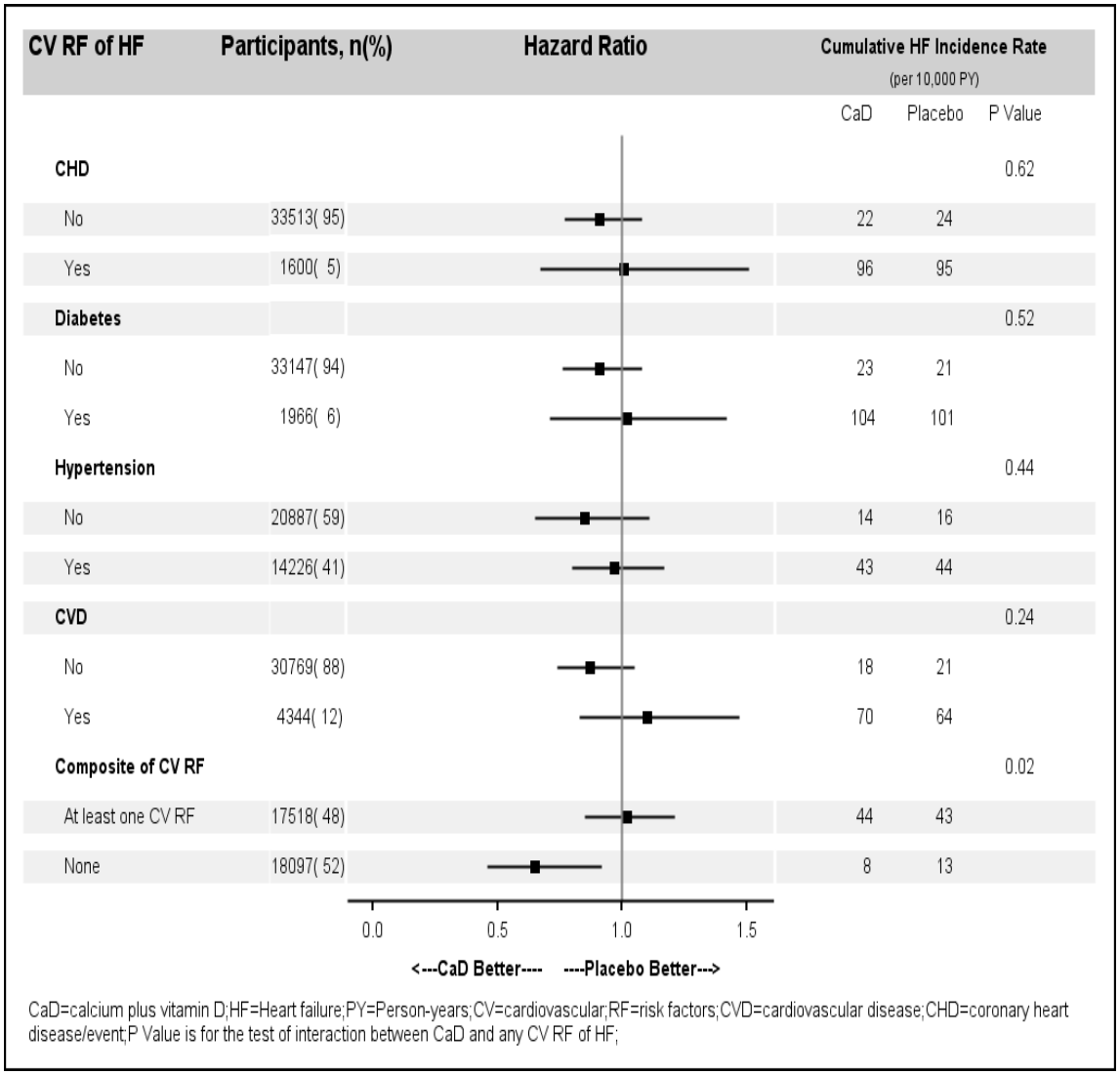
Association between calcium (1000 mg/day) and vitamin D (400 IU/day) supplementation and heart failure stratified by baseline preexisting diagnosed cardiovascular risk factors of heart failure, WHI study, 1995 – 2005.

Stratified by baseline HF risk factors	HF cases, n (annualized %)		Unadjusted		Fully-adjusted[†]	
	Intervention	Control	HR (95%CI)	P	HR (95%CI)	P
Hypertension [a]						
No (n= 20,887)	103 (0.14)	118 (0.16)	0.86 (0.66 – 1.12)	0.28	0.85 (0.65 – 1.11)	0.24
Yes (n= 14,226)	215 (0.43)	221 (0.44)	0.97 (0.80 – 1.17)	0.75	0.97 (0.80 – 1.17)	0.72
CVD [b]						
No (n= 30,769)	175 (0.18)	199 (0.21)	0.88 (0.73 – 1.05)	0.14	0.87 (0.74 – 1.05)	0.16
Yes (n= 4,344)	102 (0.70)	97 (0.64)	1.07 (0.83 – 1.39)	0.59	1.10 (0.83 – 1.47)	0.60
CHD/coronary [c]						
No (n= 33,513)	266 (0.22)	287 (0.24)	0.92 (0.78 – 1.09)	0.32	0.91 (0.77 – 1.08)	0.27
Yes (n=1,600)	52 (0.96)	52 (0.95)	1.01 (0.69 – 1.48)	0.96	1.01 (0.67 – 1.51)	0.97
Diabetes [d]						
No (n= 33,147)	249 (0.21)	272 (0.23)	0.91 (0.77 – 1.08)	0.27	0.91 (0.76 – 1.08)	0.27
Yes (n=1,966)	69 (1.04)	67 (1.01)	1.02 (0.73 – 1.42)	0.92	1.01 (0.71 – 1.42)	0.98
CV risk factors [e]						
None (n=18,097)	55 (0.08)	84 (0.13)	0.65 (0.46 – 0.91)	0.01	0.65 (0.46 – 0.92)	0.01
Composite (n=17,016)	263 (0.44)	255 (0.43)	1.03 (0.87 – 1.23)	0.73	1.02 (0.85 – 1.21)	0.87

Abbreviations: HF, heart failure; HR, hazard ratio; CI, confidence interval; P, p-value; CVD, cardiovascular disease; CHD, coronary heart disease

[†] stratified on clinical trial (HT/DM) and adjusted for: age, race, educational level, income, solar irradiance, BMI, physical activity, systolic blood pressure, diastolic blood pressure, calcium supplements intake, vitamin d supplement intake, use of hormone replacement therapy cholesterol levels, smoking status, alcohol intake, multivitamin use, and family history of CVD

[a] adjusted for history of CVD, history of CHD, history of diabetes and all variables in [†]
[b] adjusted for history of hypertension, history of CHD, history of diabetes and all variables in [†]
[c] adjusted for history of hypertension, history of CVD, history of diabetes and all variables in [†]
[d] adjusted for history of hypertension, CVD, CHD and all variables in [†]
[e] adjusted for all variables in [†] except history of hypertension, CVD, CHD, and diabetes



CaD=calcium plus vitamin D;HF=Heart failure;PY=Person-years;CV=cardiovascular;RF=risk factors;CVD=cardiovascular disease;CHD=coronary heart disease/event;P Value is for the test of interaction between CaD and any CV RF of HF;

Figure 16. Forest plot of hazard ratios for effect of vitamin D plus calcium supplementation on heart failure.

4.2.4 Interaction between Intervention and Baseline Self-Reported Total (diet plus supplements) Vitamin D and Calcium Intake

An interaction term between baseline total vitamin D intake (from diet and supplements) and CaD was included in both unadjusted and fully-adjusted Cox

regression models. These interaction terms were not statistically significant in either the unadjusted (*P*-interaction = 0.26) or fully-adjusted (*P*-interaction = 0.22) models suggesting total vitamin D intake did not modify the effect of CaD on the incidence of HF in the entire cohort. We also tested whether only self-reported consumption of personal vitamin D supplements (≤ 600 IU/day) modified the relationship between CaD and incidence of HF. No statistically significant interaction effect was observed between only self-reported vitamin D supplement intake and the intervention status (*P*-interaction = 0.33) in the unadjusted model. Hazard ratios were estimated for groups stratified by levels of self-reported total vitamin D intake and by only self-reported vitamin D supplement intake (Table 13). Baseline total vitamin D intake did not also modify the association between CaD and heart failure in groups stratified by baseline preexisting cardiovascular risk factors (results not reported).

Multiplicative interaction between baseline total calcium intake (from diet and supplements) and CaD was neither detected in unadjusted (*P*-interaction = 0.81) nor in fully-adjusted models (*P*-interaction = 0.53). Baseline self-reported consumption of calcium supplements (≤ 1,000 mg/day) alone did not also modify the association between the intervention and heart failure (*P*-interaction = 0.14) in the unadjusted model. Stratified hazard ratios by baseline total calcium intake and only self-reported calcium supplement intakes are reported in Tables 13. Baseline total calcium intake did not also modify the association between CaD and heart failure in groups stratified by baseline preexisting cardiovascular risk factors (results not reported).

Table 13

Association between calcium (1000 mg/day) plus vitamin D (400 IU/day) supplementation and heart failure stratified by baseline vitamin D and calcium intakes, WHI study, 1995 – 2005.

Baseline vitamin D and calcium intakes	HF cases, n (annualized %)		Unadjusted	
	Intervention	Control	95% CI	*P*
Total vitamin D (IU/day) from diet and supplements				
<200 (n=15,756)	142 (0.25)	154 (0.27)	0.91 (0.72 – 1.14)	0.42
200–600(n=14,609)	128 (0.25)	150 (0.29)	0.85 (0.67 – 1.08)	0.17
≥600 (n=4,828)	48 (0.28)	35 (0.21)	1.35 (0.89 – 2.13)	0.15
Vitamin D supplements alone				
No (n= 33,719)	309 (0.26)	325 (0.27)	0.94 (0.81 – 1.10)	0.47
Yes (n= 1,394)	9 (0.18)	14 (0.28)	0.62 (0.27 – 1.42)	0.26
Calcium (mg/day) from diet and supplements				
<800 (n=18,252)	173 (0.26)	182 (0.28)	0.93 (0.76 – 1.15)	0.50
800-1200 (n=6,899)	66 (0.26)	69 (0.28)	0.94 (0.67 – 1.31)	0.70
≥1,200 (n=9,962)	79 (0.23)	88 (0.24)	0.92 (0.68 – 1.25)	0.60
Calcium supplements alone				
No (n= 28,647)	270 (0.26)	274 (0.27)	0.98 (0.83 – 1.16)	0.82
Yes (n= 6,466)	48 (0.21)	65 (0.29)	0.72 (0.50 – 1.05)	0.09

Abbreviations: HF, heart failure; HR, hazard ratio; CI, confidence interval; *P*, *P*-value

4.3 Sensitivity Analyses

3.3.1 Per-protocol Analyses

During the intervention phase of the study, 57.6% (n = 20,227) of the participants adhered to at least 80% of the study medication; the placebo group had a slightly higher 80% adherence rate, 58.8% (n = 10,293) compared to the intervention group, 56.5% (n = 9,934). Chi-square tests and t-tests of the distribution of baseline covariates between

study arms indicated all covariates but smoking status ($P = 0.05$) were balance. However, owing to the 42.4% reduction of the original study sample, only known demographic (age, race, and educational status), lifestyle/physical (BMI, physical activity, systolic and diastolic blood pressures), and cardiovascular risk factors (hypertension, CVD, CHD, and diabetes) were included in multivariable Cox proportional hazard (CPH) regression models. Smoking status was included in these models because it is an important risk factor of HF and also for not being proportionally distributed between the intervention and placebo groups. Hazard ratios were estimated for the entire study sample and also by the two groups stratified by preexisting cardiovascular risk factors of HF. Similar to the intention-to-treat analyses, CaD was associated with a 5% (HR = 0.95, 95% CI:0.75 – 1.19) and 42% (HR = 0.58, 95% CI:0.35 – 0.96) reduced risk of heart failure incidence in the entire CaD cohort and in populations without preexisting baseline cardiovascular risk factors of heart failure respectively (Table 14). Again, CaD was associated with a small elevated risk of heart failure, 5% (HR = 1.05, 95% CI:0.81 – 1.37) in populations with preexisting cardiovascular risk factors of heart failure (Table 13).

Table 14

Association between calcium (1000 mg/day) and vitamin D (400 IU/day) supplementation and heart failure among the study population with at least 80% adherence to study medication, WHI study, 1995 – 2005.

Population	HF cases, n (annualized %)		HR (95% CI)	*P*	*P*-interaction
	Intervention	Control			
Entire CaD cohort (n=20,227)	137 (0.19)	155 (0.21)	0.95 (0.75 – 1.19)	0.66	NA
Stratified by baseline CV risk factors					
None (n= 10,736)	24 (0.06)	41 (0.10)	0.58 (0.35 – 0.96)	0.03	
Composite (n= 9,491)	113 (0.34)	114 (0.33)	1.05 (0.81 – 1.37)	0.69	<0.001

Abbreviations: CaD, calcium plus vitamin D trial; CV, cardiovascular; HF, heart failure; HR, hazard ratio; CI, confidence interval; *P*, p-value for HR; *P*-interaction, p-value for interaction

4.3.2 Estimating Hazard Ratios Independent of Noncompliance to Study Protocol by Inverse Probability of Censored Weights (IPCW) Method

The hazard ratios for the intervention estimated via the inverse probability of censored weights (IPCW) method are reported in Table 15. The intervention was still observed to be associated with reduced risk of heart failure in the CaD cohort and in populations without preexisting baseline cardiovascular risk factors of heart failure. The intervention was also still not associated with risk of heart failure in populations with preexisting baseline cardiovascular risk factors of heart failure.

Table 15

Estimated hazard ratios for the association between the intervention and risk of heart failure independent of competing risks and noncompliance to study medication through the inverse probability of censored weights method (IPCW).

Population	HR (95% CI)			
	Unadjusted	***P***	**Adjusted***	***P***
Entire CaD cohort (n=35,113)	0.90 (0.71 – 1.15)	0.40	0.90 (0.71 – 1.15)	0.41
Stratified by baseline CV risk factors				
None (n=18,097)	0.65 (0.35 - 1.21)	0.18	0.65 (0.34 – 1.23)	0.19
Composite (n = 17,016)	1.01 (0.81 – 1.26)	0.91	1.02 (0.82 – 1.26)	0.90

Abbreviations: CaD, calcium plus vitamin D trial; CV, cardiovascular; HF, heart failure; HR, hazard ratio; CI, confidence interval; *P*, p-value for HR

*adjusted for – age, education, use of personal calcium, vitamin D or multivitamin supplements, history of heart failure risk factors (hypertension, CVD, CHD/coronary events, or diabetes), family history of CVD, and enrollment in other clinical trials

4.3.4 Interaction between Intervention and Baseline Serum 25(OH)D Levels

The mean (±SD) baseline serum 25-hydroxyvitamin D concentrations were similar between the intervention (17.3 ± 5.2 ng/mL) and placebo (16.7 ± 5.4 ng/mL) groups, $P = 0.44$. Baseline 25-hydroxyvitamin D concentrations also did not significantly interact with the intervention, P-interaction = 0.45.

CHAPTER 5

DISCUSSION

5.1 Summary of Results

In the calcium plus vitamin D trial of the Women's Health Initiative (WHI) study, 318 of the 17,595 participants randomized to 1,000 mg calcium plus 400 IU vitamin D_3 per day (CaD) had a first (incident) hospitalization for heart failure (HF) during an average follow-up time of 7.13 (SD, 1.33) years, while 339 of the 17,518 participants randomized to control had a first hospitalization for HF during the same period. The crude annual hazard rate of HF in the control group was slightly higher than that of the intervention group (27.1 vs. 25.2/10,000 person-years). CaD was associated with a 7% reduced risk of HF which increased slightly to 8% after adjusting for HF demographic, lifestyle/behavioral, and cardiovascular disease risk factors in multivariable-adjusted Cox proportional hazards (CPH) regression models. With an a priori power of 80% and an alpha level of 0.05, this study could not reject the null hypothesis of no CaD effect for a hazard ratio (HR) of 0.93 (> the critical HR, 0.83) among 35,113 postmenopausal women.

It was also observed that this association was modified by baseline risk status of HF. When the study population was stratified by baseline preexisting diagnosed

cardiovascular risk factors of HF, the intervention was significantly associated with a 35% risk reduction of HF among participants without preexisting diagnosed cardiovascular risk factors of HF (hypertension, cardiovascular diseases, coronary heart diseases/events, or diabetes) at baseline. On the other hand, the CaD was not associated with risk of HF (HR = 1.02, 95% CI:0.85 – 1.21) among the participants with these risk factors. These observed associations were neither modified by baseline total (diet and supplements) calcium nor vitamin D intake. Even though participants were allowed to continue consumption of personal calcium and/or vitamin D supplements, this did not modify the association between the intervention and HF.

In sensitivity analyses, CaD still did not significantly reduce the risk of HF (HR = 0.95, 95% CI:0.75 – 1.19) among the 20,227 (57.6% of the CaD cohort) participants who were at least 80% adherent to the study protocol. When this group was stratified by baseline diagnosed cardiovascular risk factors of HF, CaD was associated with a greater and significant reduction of risk of HF, 43%, in the populations without preexisting baseline diagnosed cardiovascular risk factors. The 80% adherence rate to study medication did not change the association between the intervention and HF in populations with preexisting baseline cardiovascular risk factors. The HRs estimated for the association between the intervention and time-to-HF in the Cox proportional hazard regression models in the main analyses remained unchanged even after adjusting for noncompliance to study medication through the inverse probability of censoring weights (IPCW) method. The effect of CaD on HF incidence estimated in the main and sensitivity analyses were similar (in magnitude and statistical significance) to those estimated

involving the entire original CaD cohort of 36,282 postmenopausal women (results not shown).

These results suggest that while supplementation with calcium plus vitamin D did not significantly reduce the risk of HF among all postmenopausal women, preexisting cardiovascular risk factors of HF may modify this association. Hence, among participants without any preexisting diagnosis of cardiovascular risk factors (hypertension, CVD, CHD/events, or diabetes), CaD may cut the risk of HF by as much as 35% during an average follow-up time of about 7 years. However, among postmenopausal women with these risk factors, CaD may not be associated with any benefits for HF. This implies the intervention may be a good primary preventive agent for HF since it was associated with strong significant risk reduction in a group of women without stage A or B HF (those without preexisting hypertension, CVD, coronary heart disease/events, or diabetes) but not in those with stage A or B HF (those with preexisting hypertension, CVD, coronary heart disease/events, or diabetes). There are no known existing data to compare the findings of this study with. Plausible competing explanations for these findings are discussed below.

5.1.1 Effect of Intervention in the Overall CaD Cohort

The lack of a strong statistically significant effect of the intervention in the overall CaD cohort could be explained by the following theories:

1. Effect modification by baseline risk status: There was strong evidence of statistical interaction between the baseline risk status and the intervention in both unadjusted and multivariable adjusted CPH regression models. Qualitative interaction was observed

because the intervention was associated with both significant and lower risk of HF in women free of stage A or B HF but associated with a small elevated non-significant (2%, $P = 0.87$) risk of HF in those with stage A or B HF. This suggests there is heterogeneity in the effect of intervention on the risk of HF that is conditional on the existence of HF precursors.

In combination and individually, preexisting diagnosed cardiovascular risk factors at baseline were not confounders in this study. Individually, hypertension, CVD, CHD/events, and diabetes were equally distributed between the intervention and control groups (Table 6). When combined, they were still not significantly associated with the randomization status, χ^2 (0.49), $P = 0.49$. In order for the baseline risk status to be considered a confounder it must be associated with both the outcome, HF, and the exposure, randomization status. While the composite of these risk factors was associated with HF, empirically it is not associated with the randomization.

Because the composite of preexisting diagnosed cardiovascular risk factors at baseline was ruled out as a confounder and evidence suggests it was an effect modifier, it is conceivable to observe both weak and nonsignificant hazard ratios associated with CaD in the overall trial sample without stratification. In the presence of an effect modification, the overall hazard ratio could be misleading and at best, noninformative.

2. Heart failure (HF) misclassification bias: The main event, heart failure incidence, was based on self-reported hospitalization for HF. These reports were then adjudicated by a trained physician committee at the local trial site. Even though the adjudication process improves the validity of HF cases, asymptomatic HF patients and those with pre-clinical

HF who were not yet being hospitalized for HF were not identified as cases. This underestimation of HF incidence was likely to be equal in both study arms. That is, it is unlikely there would be differential misclassification bias because both study participants and the adjudication committee were blinded to the treatment allocation. The underestimation of the incidence of HF in both study arms at equal proportions would have attenuated the estimated hazard ratio which could explain the small risk reduction of HF observed in this study.

3. Low vitamin D doses: The dosage of vitamin D in the CaD trial was previously suggested by Hsia et al. (2007) as a plausible explanation for lack of lack of observed strong association between the intervention and risk of cardiovascular events (129). The current RDA for vitamin D is 600 IU/day for persons below 70 years old but the intervention consisted of only 400 IU/day of vitamin D. While the revised RDA for vitamin D, 600 IU/day, was aimed at raising serum 25(OH)D levels to optimize bone health, it is unclear if such a dose might be adequate as an effective agent for a non-musculoskeletal events such as HF. This subject will continue to be an open debate until trials are designed to specifically test the dose-response relationship between vitamin D and cardiovascular events in the general population.

4. Possible calcium antagonistic effects: The role of calcium in cardiovascular disease and heart failure pathogeneses is still not clear. While there are no existing data on the effect of calcium supplementation, with/without vitamin D, on HF, meta-analyses of trials evaluating the effect of such an intervention on other cardiovascular diseases have either reported elevated or no risk (5, 8, 186). Given these findings, it is plausible calcium

did not play a significant role on the association observed between the intervention and HF in the current study. However, if calcium supplementation is associated with elevated risk of HF, then it is likely calcium had antagonistic effect against any potential protective effect of vitamin D against HF, and hence pull the effect estimate towards the null as observed, HR = 0.92. The CaD trial data does not support the theory that calcium may be associated with elevated risk of HF. Neither total (diet plus supplements) calcium nor calcium supplements (only personal supplements) modified the association between the intervention and risk of HF in the analyses of the CaD trial data. Hence, it is likely the observed small (8%) nonsignificant risk reduction of HF was not a result of combining vitamin D with calcium for the intervention in the CaD trial. Hence, it is unlikely that calcium attenuated the effect of vitamin D on HF in this study.

5.1.2 Effect of the Intervention in Populations with Preexisting Baseline Cardiovascular Risk Factors of Heart Failure

Contrary to the second primary hypothesis of this study, CaD will be associated with stronger lower risk of HF in preexisting baseline cardiovascular risk factors, the association of the intervention with risk of HF was null and nonsignificant in this group. There was also no evidence to reject the null hypothesis in favor of our alternate hypothesis. While this finding may also appear to contradict existing literature that support a beneficial role for vitamin D in heart failure prevention, several reasons could help explain the finding of this study.

One plausible explanation for the lack of an association between CaD and risk of HF in this population is that they were more predisposed to developing heart failure

compared to those without the cardiovascular risk factors of heart failure. This could have diminished any benefit that might have been achieved towards HF prevention. Our data suggests that the stage A or B HF group indeed might be of poorer health status compared to those free of stage A or B HF with regards to heart failure incidence. Other factors associated with poor health and risk of HF such as older age, educational status, obesity, and hypercholesterolemia were significantly higher in the stage A or B HF group to their counterparts (Table 16). The notable exception is smoking (current or past), a risk factor of HF, which was rather higher in those without stage A or B HF – perhaps, those in stage A or B HF group were asked by their health care givers to quit smoking owing to their health status.

Mortality was significantly higher in the stage A or B HF group (Table 16). This is another indication of the relatively poorer health status of this population compared to those free of stage A or B HF. Death could therefore have been a competing risk event in the estimation of hazard ratios (HRs) for the intervention from the Cox proportional hazard regression models. That is, death as a competing risk prevents the incidence of heart failure which masks the effect of the intervention on heart failure. However, in the stage A or B HF group, there was not a significant difference ($P = 0.09$) in mortality between the intervention (n = 464, 47.3%) and control (n = 517, 52.7%) groups. Additionally, the HRs did not change when estimated independent of competing events and noncompliance to study protocol through the inverse probability of censoring weights (IPCW) method; the HR associated with the intervention increased marginally from 1.02 to 1.05 (Table 15).

Table 16

Distribution of selected baseline covariates by the presence and absence of baseline cardiovascular risk factors of heart failure

Risk factors	**Preexisting baseline cardiovascular risk factors of heart failure**		**P-value**
	Absent	**Present**	
Age (mean ± SD)	61.1 (6.7)	63.6 (6.9)	
50 – 59	7,995 (44.2)	5,050 (48.1)	<0.001
60 – 69	7,799 (43.1)	8,176 (48.1)	
70 - 81	2,303 (12.7)	3,790 (22.3)	
Smoking			
Never	9,491 (52.5)	9,069 (53.3)	< 0.001
Past	7,140 (39.5)	6,752 (39.7)	
Current	1,466 (8.1)	1,195 (7.0)	
BMI (kg/m^2)			
<24.9	6,420 (35.5)	3,567 (21.0)	<0.001
25.0 – 29.9	6,656 (36.8)	5,794 (34.1)	
≥30.0	5,021 (27.7)	7,655 (45.0)	
Education			
None/grade school	319 (1.8)	406 (2.4)	<0.001
High school/some college	10,565 (58.4)	11,105 (65.3)	
College	2,013 (11.1)	1,576 (9.3)	
Beyond college	5,200 (28.7)	3,929 (23.1)	
Vitamin D supplements	7,563 (41.8)	6,652 (39.1)	< 0.001
Calcium supplements	8,793 (48.6)	7,680 (45.1)	< 0.001
Multivitamin supplements	6,276 (34.7)	5,909 (34.7)	0.93
Hypercholesterolemia	1,209 (6.7)	2,582 (15.2)	<0.001
Adherence rate	7,361 (40.7)	7,525 (44.2)	<0.001
Mortality	432 (2.4)	981 (5.8)	<0.001

It is also plausible that medical therapy for treatment of the cardiovascular risk factors in the stage A or B HF group might have reduced the incidence of HF thereby rendering the intervention impotent. Interaction between the intervention and cardiovascular disease medications (ACE inhibitors, statins, beta blockers, and calcium blockers) was evaluated in sensitivity analysis (Supplementary tables). None of these medications, individually or their composite, interacted significantly with the intervention at statistical significant alpha level of 0.10. However, in the group free of stage A or B HF there was significant interaction between the use of ACE inhibitors (n = 1,006; 5.4%) and the intervention, *P*-interaction = 0.05. The intervention was associated with about a 2-fold increased significant risk of HF (HR = 1.94, 95% CI:1.32 – 2.84) among users of ACE inhibitors but non-significant lower risk (HR = 0.93, 95% CI:0.50 – 1.72) among non-users in group free of stage A or B HF. In the group with stage A or B HF, only the use of calcium blockers (n = 5,691; 32.6%) had a significant interaction effect on the intervention, *P*-interaction = 0.09. The effects of the intervention stratified by use of calcium blockers were not statistically significant, however, a lower risk was observed among non-users (HR = 0.83, 95% CI:0.66 – 1.04) compared to an elevated risk among users (HR = 1.11, 95% CI:0.87 – 1.38).

It appears therapeutic use of ACE inhibitors in the group of women without preexisting hypertension, CVD, CHD/events or diabetes modified the effect of CaD on HF. This suggests that the therapeutic use of ACE inhibitors for other medical conditions besides hypertension, CVD, CHD/events or diabetes helps reduce the risk of HF among women taking CaD supplements. Calcium channel blockers, another high blood pressure control medication, also appeared to modify the relationship between CaD and HF; CaD

increased the risk of HF among users of calcium channel blockers. This suggests calcium channels blockers may have an antagonistic effect on the association between CaD and HF since a lower risk was observed in non-users but not in users. It could also mean that calcium channels blockers do not have any antagonistic effect and that the elevated HF risk observed is a result of the effect of the advanced stage of the conditions that are being treated with calcium channel blockers. Based on these results it is unclear what role cardiovascular therapy played in the null effect observed for the association between CaD and HF.

In the absence of confounders, especially those not observed and/or controlled for in the analyses, the findings in the stage A or B HF group suggests CaD was not an effective secondary prevention therapy in among women with stage A or B heart failure. However, CaD may still be associated with HF through a direct pathway as suggested by data from women without stage A or B HF.

5.1.3 Effect of the Intervention in Populations without Preexisting Baseline Cardiovascular Risk Factors of Heart Failure

The intervention was associated with a lower risk of HF, compared to placebo, in both unadjusted and multivariable-adjusted Cox proportional hazard regression models in the primary as well as sensitivity analyses in a population of postmenopausal women free of prevalent HF and the major HF cardiovascular risk factors. Calcium plus vitamin D supplementation was associated with a 35% HF risk reduction during an average follow-up period of 7.13 (SD, 1.33) years. Several competing explanations for this finding are discussed.

Biological explanations for this association relate to the role of vitamin D in the pathogenesis of HF. While data from animal models suggest a direct causal pathway between low vitamin D and HF, both animal and human studies also suggest an indirect causal pathway model involving the up-regulation of the renin-angiotensin-aldesterone system (RAAS), and hyperparathyroidism (110).

Data from animal models suggest vitamin D may regulate cardiac functions at least partially via interaction with the vitamin D receptor (VDR) in cardiac myocytes (10, 119). Through a receptor-mediated mechanism, $1,25(OH)_2D$ regulates intracellular calcium homeostasis and calcium ion uptake in ventricular cardiac muscle cells to modify cardiac contractility (120). While this phenomenon at the cellular level could not be observed in the CaD trial or other human studies, it is plausible that the reduced risk of HF associated with calcium plus vitamin D supplementation was achieved in this manner.

The intermediate model involving the up-regulation of RAAS has been observed in both human and animal studies. The renin-angiotensin-aldosterone system (RAAS) plays a major role in HF pathogenesis by regulating blood pressure, cardiac contractility, electrolyte homeostasis, and eccentric hypertrophy of the myocardium (111, 112). It has been demonstrated that mice without the vitamin D receptor (VDR) developed high blood pressure, cardiac enlargement, and experience increased activation of the RAAS (113). The activation of RAAS by low vitamin D status has recently been suggested in humans based on observational studies (114, 115). However, a meta-analysis of 10 trials, including the WHI's vitamin D and calcium trial, did not show an association between

vitamin D (alone or with calcium) supplementation and reduction in either systolic nor diastolic blood pressures (6).

Hyperparathyroidism has also been postulated as an intermediate process in the vitamin D-heart failure pathophysiology. Data from end-stage renal disease (ESRD) patients suggests secondary hyperparathyroidism and elevated parathyroid hormone (PTH) levels were associated with low vitamin D levels, caused by failure of the kidneys to convert 25(OH)D to the metabolic form of $1,25(OH)D_2$ (10, 15-17). Chronic exposure to PTH has been reported to be associated with poor myocardial structure and functioning as well as elevated blood pressure and accelerated atherosclerosis. Excess PTH may also lead to cardiomyocyte hypertrophy, an important risk factor for HF, especially among hemodialysis patients (13, 15, 118). Therefore, supplementation with both vitamin D and calcium could have prevented or mitigated hyperparathyroidism and subsequently reduced HF incidence in the intervention group.

Another indirect causal pathway involves pancreatic beta cell dysfunction and insulin resistance both of which are major risk factors of type 2 diabetes and the metabolic syndrome. Both type 2 diabetes and the metabolic syndrome can lead to heart failure (110). Vitamin D was observed to induce insulin secretion by the pancreas in animal models (187). Hyperparathyoridsm have also been implicated in pancreatic beta cell dysfunction and disruption of normal insulin secretion in rats (188). However, vitamin D supplementation, with or without calcium, had no significant effect on fasting plasma glucose level in populations with normal glucose tolerance at baseline in a meta-analysis of 5 trials (6). Additionally, in the WHI CaD trial, calcium plus vitamin D

supplementation was not associated with the incidence of type 2 diabetes (189). It is therefore not clear if the lower risk reduction of heart failure associated with calcium plus vitamin D supplementation in our study involved this pathway, given these equivocal findings.

The data presented above suggests the association between vitamin D plus calcium supplementation and heart failure incidence may be a biological one. Specifically, a direct pathophysiological between vitamin D and heart failure pathogenesis appears to be supported by this study. Evidence from this study is not sufficient to support the indirect pathophysiological model because of the lack of association between CaD and heart failure incidence among postmenopausal women with preexisting diagnosed cardiovascular risk factors of heart failure. The randomized study design also allows us to establish temporality for this association between CaD and incidence of heart failure, that is the potential putative factor, CaD supplementation, precedes the incidence of heart failure. The statistically and clinically significant effect of CaD on heart failure incidence in this population in both intention-to-treat and per-protocol analyses implies the intervention was both effective and efficacious.

5.2 Limitations

Study limitations are inherent in the original study design of the vitamin D and calcium (CaD) trial. First, As a result of combining calcium with vitamin D as the intervention, it is not clear whether the observed associations are due to either just one of these chemical agents or both. Second, the trial was limited to postmenopausal women, 50 – 81 years old, which limits the ability to generalize the findings to younger females

and males of all ages in the general population. Third, heart failure (HF) incidence was based on the participant's self report of being hospitalized for HF would have probably underestimated the true incidence since participants with pre-clinical or asymptomatic HF would not have been captured. However, this type of bias is expected to be non-differential and if eliminated, would result in stronger effect estimates away from the null. Lastly, for ethical reasons, participants were allowed to continue consumption of both vitamin D and calcium supplements within the recommended dietary allowance guidelines by the Institute of Medicine. However, neither baseline total (diet plus supplements) calcium nor vitamin D intake modified the association between the intervention and risk of HF. Baseline vitamin D and calcium supplements (only) intake did not also modify the association between the intervention and risk of HF.

5.3 Strengths

This study contributes to the growing literature on the association between vitamin D and incidence of heart failure (HF). This study is the first to demonstrate a significant HF risk reduction associated with vitamin D, with calcium, supplementation among postmenopausal women. The study also demonstrated that while vitamin D, with calcium, supplementation may be associated with reduced risk of HF, this association may only exist among postmenopausal women without preexisting diagnoses of cardiovascular risk factors of HF (hypertension, cardiovascular diseases, coronary heart diseases/events, or diabetes). The study had adequate statistical power to detect the observed hazard ratio of 0.65 associated with CaD in the sample of postmenopausal women free of HF and diagnoses of cardiovascular risk factors at baseline. The study was

randomized to minimize confounding effects by other covariates associated with both vitamin D status and HF. All selected covariates were balanced between study arms for the entire CaD cohort and the stratified cohort based on preexisting major HF cardiovascular risk factors at baseline.

5.4 Public Health Implications

These findings, if confirmed in other research, have important public health and clinical implications. Findings from this study suggests that the risk of heart failure, a major public health concern, can be reduced by a simple and inexpensive intervention of calcium plus vitamin D supplementation among postmenopausal women who do not have preexisting major heart failure risk factors such as hypertension, cardiovascular diseases, coronary heart diseases/events, or diabetes. In this population, supplementation with calcium plus vitamin D for a period of about 7.5 years may prevent one heart failure event among approximately 250 individuals (Figure 14).

Specifically, this low-cost (about $35.00 annually) public health prevention strategy for heart failure which costs about $17,654.00 to $25,325.00 per hospitalization could be a very cost-effective one (190, 191). To illustrate how much healthcare cost for treatment of heart that can be saved with vitamin D plus calcium supplements, it costs about $65,625.00 (=250[NNT] x 7.5 [years of supplementation] x $35.00[cost of supplements/year]) to prevent one heart failure from occurring in a postmenopausal woman without CV risk factors of HF a 7.5 year period through vitamin D plus calcium supplementation. However, if this individual woman had developed HF, it would have cost about $132,405.00 to $189,937.50 to treat her in the same period of time, 7.5 years,

assuming 1 hospitalization per year. The trade off is clear, about $66,780 to $124,312.50 could be saved in the cost of hospitalization of heart failure alone for this woman.

In addition to the reduction in cost of hospitalization, other healthcare costs due to the treatment and management of heart failure such as medication, home healthcare, physicians, and nursing home care could be saved with this inexpensive intervention. Additionally, this inexpensive intervention could also save this woman from the enormous and invaluable physical and emotional burden of heart failure the ultimately prevented a premature death from heart failure.

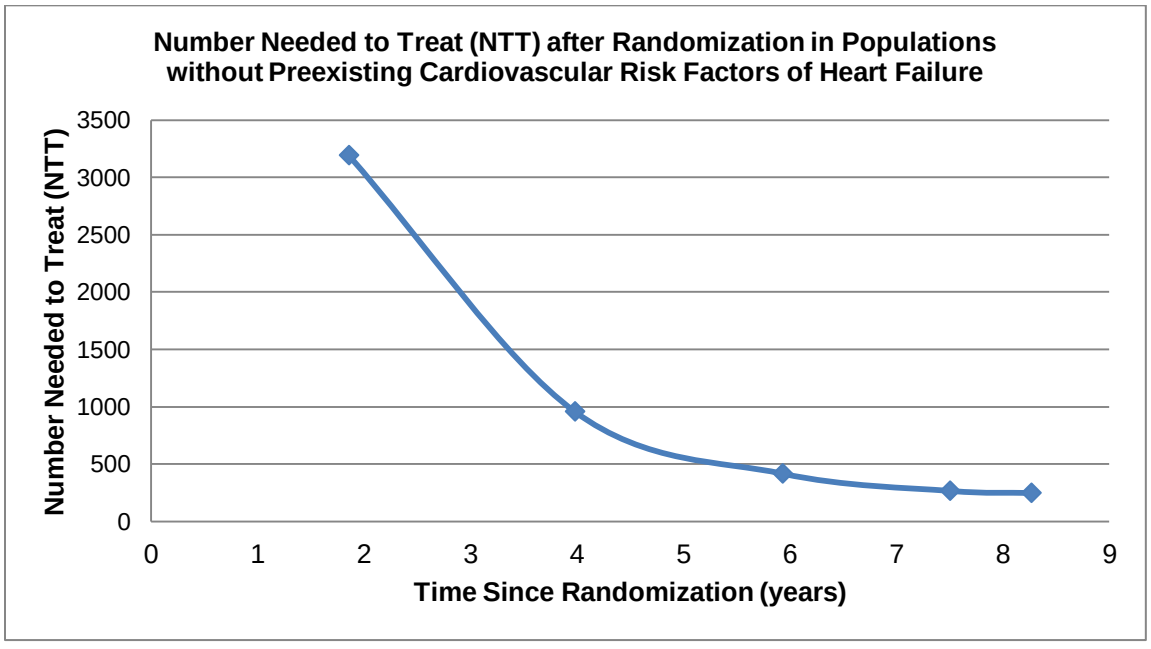


Figure 17. Plot of number needed to treat by vitamin D and calcium supplementation in populations without preexisting cardiovascular risk factors of heart failure.

CONCLUSION

Calcium (1000 mg/day) plus vitamin D (400 IU/day) supplementation was associated with a nonsignificant reduction of risk of heart failure among postmenopausal women enrolled in the vitamin D and calcium trial (CaD) of the Women's Health Initiative (WHI) study during an average follow-up period of 7 years. However, this association was modified by the presence of preexisting diagnosed cardiovascular risk factors of heart failure (hypertension, cardiovascular diseases, coronary heart disease/events, or diabetes) at baseline. CaD was associated with a 35% statistically significant risk reduction of heart failure among the sample without preexisting diagnosed cardiovascular risk factors of heart failure. Among women with these cardiovascular risk factors at baseline, CaD was not associated with risk of heart failure. While the analyses confirmed one of our study primary hypotheses, that baseline risk status of heart failure modified the association between the intervention and risk of heart failure, there was not strong evidence to reject the null hypothesis that the intervention

was not associated with risk of heart failure in the overall CaD cohort. Additionally, contrary to the study's hypothesis that the intervention was associated with a stronger risk reduction of heart failure in the population with preexisting baseline cardiovascular risk factors of heart failure than in the population without these risk factors, stronger risk reduction was rather observed in the latter population. Future studies are warranted to replicate our findings.

RECOMMENDATIONS

Future intervention trials of vitamin D supplements, both with and without calcium, should be conducted in the general adult male and female populations. The population heterogeneity of baseline risk status for heart failure should be considered when designing future trials. It will also be important to design future trials in a manner that will allow investigators to estimate the association between heart failure incidence and vitamin D alone, calcium alone, or their combination. A 2 x 2 multifactorial trial design calcium plus vitamin D, calcium alone, vitamin D alone, and placebo is one design that will help future investigators estimate the association between these vitamins and heart failure. It may also be important to use dose of vitamin D of at least 600 IU/day in future trials, according to the current IOM's recommendation. Above all, future studies should pre-specify heart failure as a primary outcome of interest and explicitly define it's ascertainment criteria. A central adjudication committee should always be used to certify all self-reported cases of hospitalization for heart failure as incident heart failure cases.

REFERENCES

1. Ramani GV, Uber PA, Mehra MR. Chronic heart failure: contemporary diagnosis and management. *Mayo Clin Proc* 2010;85(2):180-95.

2. Roger VL, Go AS, Lloyd-Jones DM, et al. Heart disease and stroke statistics--2011 update: a report from the American Heart Association. *Circulation* 2011;123(4):e18-e209.

3. Roger VL, Weston SA, Redfield MM, et al. Trends in heart failure incidence and survival in a community-based population. *JAMA* 2004;292(3):344-50.

4. Lloyd-Jones DM, Larson MG, Leip EP, et al. Lifetime risk for developing congestive heart failure: the Framingham Heart Study. *Circulation* 2002;106(24):3068-72.

5. Wang L, Manson JE, Song Y, et al. Systematic review: Vitamin D and calcium supplementation in prevention of cardiovascular events. *Ann Intern Med* 2010;152(5):315-23.

6. Pittas AG, Chung M, Trikalinos T, et al. Systematic review: Vitamin D and cardiometabolic outcomes. *Ann Intern Med* 2010;152(5):307-14.

7. Bjelakovic G, Gluud LL, Nikolova D, et al. Vitamin D supplementation for prevention of mortality in adults. *Cochrane Database Syst Rev* 2011(7):CD007470.

8. Chung M, Balk EM, Brendel M, et al. Vitamin D and calcium: a systematic review of health outcomes. *Evid Rep Technol Assess (Full Rep)* 2009(183):1-420.

9. Grandi NC, Breitling LP, Brenner H. Vitamin D and cardiovascular disease: systematic review and meta-analysis of prospective studies. *Prev Med* 2010;51(3-4):228-33.

10. Nemerovski CW, Dorsch MP, Simpson RU, et al. Vitamin D and cardiovascular disease. *Pharmacotherapy* 2009;29(6):691-708.

11. Rahman A, Hershey S, Ahmed S, et al. Heart extracellular matrix gene expression profile in the vitamin D receptor knockout mice. *J Steroid Biochem Mol Biol* 2007;103(3-5):416-9.

12. Simpson RU, Hershey SH, Nibbelink KA. Characterization of heart size and blood pressure in the vitamin D receptor knockout mouse. *J Steroid Biochem Mol Biol* 2007;103(3-5):521-4.

13. Achinger SG, Ayus JC. The role of vitamin D in left ventricular hypertrophy and cardiac function. *Kidney Int Suppl* 2005(95):S37-42.

14. Drueke TB, McCarron DA. Paricalcitol as compared with calcitriol in patients undergoing hemodialysis. *N Engl J Med* 2003;349(5):496-9.

15. Rostand SG, Drueke TB. Parathyroid hormone, vitamin D, and cardiovascular disease in chronic renal failure. *Kidney Int* 1999;56(2):383-92.

16. Shoji T, Shinohara K, Kimoto E, et al. Lower risk for cardiovascular mortality in oral 1alpha-hydroxy vitamin D3 users in a haemodialysis population. *Nephrol Dial Transplant* 2004;19(1):179-84.

17. Teng M, Wolf M, Ofsthun MN, et al. Activated injectable vitamin D and hemodialysis survival: a historical cohort study. *J Am Soc Nephrol* 2005;16(4):1115-25.

18. Hunt SA, Abraham WT, Chin MH, et al. 2009 Focused update incorporated into the ACC/AHA 2005 Guidelines for the Diagnosis and Management of Heart Failure in Adults A Report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines Developed in Collaboration With the International Society for Heart and Lung Transplantation. *J Am Coll Cardiol* 2009;53(15):e1-e90.

19. Kannel WB. Incidence and epidemiology of heart failure. *Heart Fail Rev* 2000;5(2):167-73.

20. Hunt SA, Abraham WT, Chin MH, et al. 2009 focused update incorporated into the ACC/AHA 2005 Guidelines for the Diagnosis and Management of Heart

Failure in Adults: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines: developed in collaboration with the International Society for Heart and Lung Transplantation. *Circulation* 2009;119(14):e391-479.

21. Dickstein K, Cohen-Solal A, Filippatos G, et al. ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure 2008: the Task Force for the Diagnosis and Treatment of Acute and Chronic Heart Failure 2008 of the European Society of Cardiology. Developed in collaboration with the Heart Failure Association of the ESC (HFA) and endorsed by the European Society of Intensive Care Medicine (ESICM). *Eur Heart J* 2008;29(19):2388-442.

22. Swedberg K, Cleland J, Dargie H, et al. Guidelines for the diagnosis and treatment of chronic heart failure: executive summary (update 2005): The Task Force for the Diagnosis and Treatment of Chronic Heart Failure of the European Society of Cardiology. *Eur Heart J* 2005;26(11):1115-40.

23. Heart Failure Society Of A. HFSA 2006 Comprehensive Heart Failure Practice Guideline. *J Card Fail* 2006;12(1):e1-2.

24. Katz AM, Zile MR. New molecular mechanism in diastolic heart failure. *Circulation* 2006;113(16):1922-5.

25. Zile MR, Brutsaert DL. New concepts in diastolic dysfunction and diastolic heart failure: Part I: diagnosis, prognosis, and measurements of diastolic function. *Circulation* 2002;105(11):1387-93.

26. Braunwald E ed. Clinical manifestations of heart failure. . Philadelphia: WB Saunders, 1980

27. Chatterjee K, Massie B. Systolic and diastolic heart failure: differences and similarities. *J Card Fail* 2007;13(7):569-76.

28. McDonagh TA, Morrison CE, Lawrence A, et al. Symptomatic and asymptomatic left-ventricular systolic dysfunction in an urban population. *Lancet* 1997;350(9081):829-33.

29. Zile MR, Baicu CF, Bonnema DD. Diastolic heart failure: definitions and terminology. *Prog Cardiovasc Dis* 2005;47(5):307-13.

30. Baicu CF, Zile MR, Aurigemma GP, et al. Left ventricular systolic performance, function, and contractility in patients with diastolic heart failure. *Circulation* 2005;111(18):2306-12.

31. How to diagnose diastolic heart failure. European Study Group on Diastolic Heart Failure. *Eur Heart J* 1998;19(7):990-1003.

32. Wang TJ, Evans JC, Benjamin EJ, et al. Natural history of asymptomatic left ventricular systolic dysfunction in the community. *Circulation* 2003;108(8):977-82.

33. Carlson KJ, Lee DC, Goroll AH, et al. An analysis of physicians' reasons for prescribing long-term digitalis therapy in outpatients. *J Chronic Dis* 1985;38(9):733-9.

34. McKee PA, Castelli WP, McNamara PM, et al. The natural history of congestive heart failure: the Framingham study. *N Engl J Med* 1971;285(26):1441-6.

35. Bui AL, Horwich TB, Fonarow GC. Epidemiology and risk profile of heart failure. *Nat Rev Cardiol* 2011;8(1):30-41.

36. Di Bari M, Pozzi C, Cavallini MC, et al. The diagnosis of heart failure in the community. Comparative validation of four sets of criteria in unselected older adults: the ICARe Dicomano Study. *J Am Coll Cardiol* 2004;44(8):1601-8.

37. Schellenbaum GD, Rea TD, Heckbert SR, et al. Survival associated with two sets of diagnostic criteria for congestive heart failure. *Am J Epidemiol* 2004;160(7):628-35.

38. Schellenbaum GD, Heckbert SR, Smith NL, et al. Congestive heart failure incidence and prognosis: case identification using central adjudication versus hospital discharge diagnoses. *Ann Epidemiol* 2006;16(2):115-22.

39. Goff DC, Jr., Pandey DK, Chan FA, et al. Congestive heart failure in the United States: is there more than meets the I(CD code)? The Corpus Christi Heart Project. *Arch Intern Med* 2000;160(2):197-202.

40. Udris EM, Au DH, McDonell MB, et al. Comparing methods to identify general internal medicine clinic patients with chronic heart failure. *Am Heart J* 2001;142(6):1003-9.

41. Labarthe D. *Epidemiology and prevention of cardiovascular diseases : a global challenge*. 2nd ed. Sudbury, Mass.: Jones and Bartlett Publishers; 2011.

42. Heckbert SR, Kooperberg C, Safford MM, et al. Comparison of self-report, hospital discharge codes, and adjudication of cardiovascular events in the Women's Health Initiative. *Am J Epidemiol* 2004;160(12):1152-8.

43. Lloyd-Jones D, Adams RJ, Brown TM, et al. Heart disease and stroke statistics--2010 update: a report from the American Heart Association. *Circulation* 2010;121(7):e46-e215.

44. McMurray JJ, Stewart S. Epidemiology, aetiology, and prognosis of heart failure. *Heart* 2000;83(5):596-602.

45. Ni H. Prevalence of self-reported heart failure among US adults: results from the 1999 National Health Interview Survey. *Am Heart J* 2003;146(1):121-8.

46. Redfield MM, Jacobsen SJ, Burnett JC, Jr., et al. Burden of systolic and diastolic ventricular dysfunction in the community: appreciating the scope of the heart failure epidemic. *JAMA* 2003;289(2):194-202.

47. Curtis LH, Whellan DJ, Hammill BG, et al. Incidence and prevalence of heart failure in elderly persons, 1994-2003. *Arch Intern Med* 2008;168(4):418-24.

48. Gottdiener JS, Arnold AM, Aurigemma GP, et al. Predictors of congestive heart failure in the elderly: the Cardiovascular Health Study. *J Am Coll Cardiol* 2000;35(6):1628-37.

49. Levy D, Kenchaiah S, Larson MG, et al. Long-term trends in the incidence of and survival with heart failure. *N Engl J Med* 2002;347(18):1397-402.

50. Hsich EM, Pina IL. Heart failure in women: a need for prospective data. *J Am Coll Cardiol* 2009;54(6):491-8.

51. Shah RU, Klein L, Lloyd-Jones DM. Heart failure in women: epidemiology, biology and treatment. *Womens Health (Lond Engl)* 2009;5(5):517-27.

52. Barker WH, Mullooly JP, Getchell W. Changing incidence and survival for heart failure in a well-defined older population, 1970-1974 and 1990-1994. *Circulation* 2006;113(6):799-805.

53. Owan TE, Redfield MM. Epidemiology of diastolic heart failure. *Prog Cardiovasc Dis* 2005;47(5):320-32.

54. Owan TE, Hodge DO, Herges RM, et al. Trends in prevalence and outcome of heart failure with preserved ejection fraction. *N Engl J Med* 2006;355(3):251-9.

55. Wolf G. The discovery of vitamin D: the contribution of Adolf Windaus. *J Nutr* 2004;134(6):1299-302.

56. Wolpowitz D, Gilchrest BA. The vitamin D questions: how much do you need and how should you get it? *J Am Acad Dermatol* 2006;54(2):301-17.

57. Hajjar V, Depta JP, Mountis MM. Q: Does vitamin D deficiency play a role in the pathogenesis of chronic heart failure? Do supplements improve survival? *Cleve Clin J Med* 2010;77(5):290-3.

58. Holick MF. Vitamin D deficiency. *N Engl J Med* 2007;357(3):266-81.

59. DeLuca HF. Overview of general physiologic features and functions of vitamin D. *Am J Clin Nutr* 2004;80(6 Suppl):1689S-96S.

60. Holick MF. Resurrection of vitamin D deficiency and rickets. *J Clin Invest* 2006;116(8):2062-72.

61. Holick MF, Binkley NC, Bischoff-Ferrari HA, et al. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab* 2011;96(7):1911-30.

62. Maxwell JD. Seasonal variation in vitamin D. *Proc Nutr Soc* 1994;53(3):533-43.

63. Millen AE, Bodnar LM. Vitamin D assessment in population-based studies: a review of the issues. *Am J Clin Nutr* 2008;87(4):1102S-5S.

64. Barragry JM, France MW, Corless D, et al. Intestinal cholecalciferol absorption in the elderly and in younger adults. *Clin Sci Mol Med* 1978;55(2):213-20.

65. Clemens TL, Zhou XY, Myles M, et al. Serum vitamin D2 and vitamin D3 metabolite concentrations and absorption of vitamin D2 in elderly subjects. *J Clin Endocrinol Metab* 1986;63(3):656-60.

66. Gray RW, Caldas AE, Wilz DR, et al. Metabolism and excretion of 3H-1,25-(OH)2-vitamin D3 in healthy adults. *J Clin Endocrinol Metab* 1978;46(5):756-65.

67. Clemens TL, Adams JS, Nolan JM, et al. Measurement of circulating vitamin D in man. *Clin Chim Acta* 1982;121(3):301-8.

68. Holick MF. Sunlight and vitamin D for bone health and prevention of autoimmune diseases, cancers, and cardiovascular disease. *Am J Clin Nutr* 2004;80(6 Suppl):1678S-88S.

69. Jacques PF, Felson DT, Tucker KL, et al. Plasma 25-hydroxyvitamin D and its determinants in an elderly population sample. *Am J Clin Nutr* 1997;66(4):929-36.

70. Giovannucci E, Liu Y, Rimm EB, et al. Prospective study of predictors of vitamin D status and cancer incidence and mortality in men. *J Natl Cancer Inst* 2006;98(7):451-9.

71. Holick MF. Vitamin D status: measurement, interpretation, and clinical application. *Ann Epidemiol* 2009;19(2):73-8.

72. Malabanan A, Veronikis IE, Holick MF. Redefining vitamin D insufficiency. *Lancet* 1998;351(9105):805-6.

73. Norman AW, Bouillon R, Whiting SJ, et al. 13th Workshop consensus for vitamin D nutritional guidelines. *J Steroid Biochem Mol Biol* 2007;103(3-5):204-5.

74. IOM 2011 Dietary reference intakes for calcium and vitamin D. Washington, DC, 2011.

75. Heaney RP, Dowell MS, Hale CA, et al. Calcium absorption varies within the reference range for serum 25-hydroxyvitamin D. *J Am Coll Nutr* 2003;22(2):142-6.

76. Thomas MK, Lloyd-Jones DM, Thadhani RI, et al. Hypovitaminosis D in medical inpatients. *N Engl J Med* 1998;338(12):777-83.

77. Holick MF, Siris ES, Binkley N, et al. Prevalence of Vitamin D inadequacy among postmenopausal North American women receiving osteoporosis therapy. *J Clin Endocrinol Metab* 2005;90(6):3215-24.

78. Chapuy MC, Schott AM, Garnero P, et al. Healthy elderly French women living at home have secondary hyperparathyroidism and high bone turnover in winter. EPIDOS Study Group. *J Clin Endocrinol Metab* 1996;81(3):1129-33.

79. Ginde AA, Liu MC, Camargo CA, Jr. Demographic differences and trends of vitamin D insufficiency in the US population, 1988-2004. *Arch Intern Med* 2009;169(6):626-32.

80. Forrest KY, Stuhldreher WL. Prevalence and correlates of vitamin D deficiency in US adults. *Nutr Res* 2011;31(1):48-54.

81. Scragg R. Vitamin D and type 2 diabetes: are we ready for a prevention trial? *Diabetes* 2008;57(10):2565-6.

82. Tangpricha V, Pearce EN, Chen TC, et al. Vitamin D insufficiency among free-living healthy young adults. *Am J Med* 2002;112(8):659-62.

83. Mithal A, Wahl DA, Bonjour JP, et al. Global vitamin D status and determinants of hypovitaminosis D. *Osteoporos Int* 2009;20(11):1807-20.

84. van Schoor NM, Lips P. Worldwide vitamin D status. *Best Pract Res Clin Endocrinol Metab* 2011;25(4):671-80.

85. Thuesen B, Husemoen L, Fenger M, et al. Determinants of vitamin D status in a general population of Danish adults. *Bone* 2012;50(3):605-10.

86. Prentice A. Vitamin D deficiency: a global perspective. *Nutr Rev* 2008;66(10 Suppl 2):S153-64.

87. Isaia G, Giorgino R, Rini GB, et al. Prevalence of hypovitaminosis D in elderly women in Italy: clinical consequences and risk factors. *Osteoporos Int* 2003;14(7):577-82.

88. Bruyere O, Malaise O, Neuprez A, et al. Prevalence of vitamin D inadequacy in European postmenopausal women. *Curr Med Res Opin* 2007;23(8):1939-44.

89. Norman AW. Sunlight, season, skin pigmentation, vitamin D, and 25-hydroxyvitamin D: integral components of the vitamin D endocrine system. *Am J Clin Nutr* 1998;67(6):1108-10.

90. Holick MF. McCollum Award Lecture, 1994: vitamin D--new horizons for the 21st century. *Am J Clin Nutr* 1994;60(4):619-30.

91. Chapuy MC, Preziosi P, Maamer M, et al. Prevalence of vitamin D insufficiency in an adult normal population. *Osteoporos Int* 1997;7(5):439-43.

92. Ross AC, Manson JE, Abrams SA, et al. The 2011 report on dietary reference intakes for calcium and vitamin D from the Institute of Medicine: what clinicians need to know. *J Clin Endocrinol Metab* 2011;96(1):53-8.

93. Dusso AS, Brown AJ, Slatopolsky E. Vitamin D. *Am J Physiol Renal Physiol* 2005;289(1):F8-28.

94. Holick MF. High prevalence of vitamin D inadequacy and implications for health. *Mayo Clin Proc* 2006;81(3):353-73.

95. Holick MF. Vitamin D for health and in chronic kidney disease. *Semin Dial* 2005;18(4):266-75.

96. Bell NH, Epstein S, Greene A, et al. Evidence for alteration of the vitamin D-endocrine system in obese subjects. *J Clin Invest* 1985;76(1):370-3.

97. Liel Y, Ulmer E, Shary J, et al. Low circulating vitamin D in obesity. *Calcif Tissue Int* 1988;43(4):199-201.

98. Wortsman J, Matsuoka LY, Chen TC, et al. Decreased bioavailability of vitamin D in obesity. *Am J Clin Nutr* 2000;72(3):690-3.

99. Holick MF. Vitamin D: importance in the prevention of cancers, type 1 diabetes, heart disease, and osteoporosis. *Am J Clin Nutr* 2004;79(3):362-71.

100. Aris RM, Merkel PA, Bachrach LK, et al. Guide to bone health and disease in cystic fibrosis. *J Clin Endocrinol Metab* 2005;90(3):1888-96.

101. Bischoff-Ferrari HA, Giovannucci E, Willett WC, et al. Estimation of optimal serum concentrations of 25-hydroxyvitamin D for multiple health outcomes. *Am J Clin Nutr* 2006;84(1):18-28.

102. Scragg R. Vitamin D and public health: an overview of recent research on common diseases and mortality in adulthood. *Public Health Nutr* 2011;14(9):1515-32.

103. Anderson JL, May HT, Horne BD, et al. Relation of vitamin D deficiency to cardiovascular risk factors, disease status, and incident events in a general healthcare population. *Am J Cardiol* 2010;106(7):963-8.

104. Melamed ML, Michos ED, Post W, et al. 25-hydroxyvitamin D levels and the risk of mortality in the general population. *Arch Intern Med* 2008;168(15):1629-37.

105. Dobnig H, Pilz S, Scharnagl H, et al. Independent association of low serum 25-hydroxyvitamin d and 1,25-dihydroxyvitamin d levels with all-cause and cardiovascular mortality. *Arch Intern Med* 2008;168(12):1340-9.

106. Ford ES, Zhao G, Tsai J, et al. Vitamin D and all-cause mortality among adults in USA: findings from the National Health and Nutrition Examination Survey Linked Mortality Study. *Int J Epidemiol* 2011;40(4):998-1005.

107. Autier P, Gandini S. Vitamin D supplementation and total mortality: a meta-analysis of randomized controlled trials. *Arch Intern Med* 2007;167(16):1730-7.

108. 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):669-83.

109. Shapses SA, Manson JE. Vitamin D and prevention of cardiovascular disease and diabetes: why the evidence falls short. *JAMA* 2011;305(24):2565-6.

110. Lavie CJ, Lee JH, Milani RV. Vitamin D and cardiovascular disease will it live up to its hype? *J Am Coll Cardiol* 2011;58(15):1547-56.

111. Eichhorn EJ, Bristow MR. Medical therapy can improve the biological properties of the chronically failing heart. A new era in the treatment of heart failure. *Circulation* 1996;94(9):2285-96.

112. Xiang W, Kong J, Chen S, et al. Cardiac hypertrophy in vitamin D receptor knockout mice: role of the systemic and cardiac renin-angiotensin systems. *Am J Physiol Endocrinol Metab* 2005;288(1):E125-32.

113. Li YC, Kong J, Wei M, et al. 1,25-Dihydroxyvitamin D(3) is a negative endocrine regulator of the renin-angiotensin system. *J Clin Invest* 2002;110(2):229-38.

114. Forman JP, Williams JS, Fisher ND. Plasma 25-hydroxyvitamin D and regulation of the renin-angiotensin system in humans. *Hypertension* 2010;55(5):1283-8.

115. Tomaschitz A, Pilz S, Ritz E, et al. Independent association between 1,25-dihydroxyvitamin D, 25-hydroxyvitamin D and the renin-angiotensin system: The Ludwigshafen Risk and Cardiovascular Health (LURIC) study. *Clin Chim Acta* 2010;411(17-18):1354-60.

116. Pilz S, Marz W, Wellnitz B, et al. Association of vitamin D deficiency with heart failure and sudden cardiac death in a large cross-sectional study of patients referred for coronary angiography. *J Clin Endocrinol Metab* 2008;93(10):3927-35.

117. Pilz S, Dobnig H, Fischer JE, et al. Low vitamin d levels predict stroke in patients referred to coronary angiography. *Stroke* 2008;39(9):2611-3.

118. London GM, Marchais SJ, Guerin AP, et al. Inflammation, arteriosclerosis, and cardiovascular therapy in hemodialysis patients. *Kidney Int Suppl* 2003(84):S88-93.

119. Witham MD. Vitamin D in chronic heart failure. *Curr Heart Fail Rep* 2011;8(2):123-30.

120. Walters MR, Ilenchuk TT, Claycomb WC. 1,25-Dihydroxyvitamin D3 stimulates 45Ca2+ uptake by cultured adult rat ventricular cardiac muscle cells. *J Biol Chem* 1987;262(6):2536-41.

121. Schleithoff SS, Zittermann A, Tenderich G, et al. Vitamin D supplementation improves cytokine profiles in patients with congestive heart failure: a double-blind, randomized, placebo-controlled trial. *Am J Clin Nutr* 2006;83(4):754-9.

122. Witham MD, Crighton LJ, Gillespie ND, et al. The effects of vitamin D supplementation on physical function and quality of life in older patients with heart failure: a randomized controlled trial. *Circ Heart Fail* 2010;3(2):195-201.

123. Zia AA, Komolafe BO, Moten M, et al. Supplemental vitamin D and calcium in the management of African Americans with heart failure having hypovitaminosis D. *Am J Med Sci* 2011;341(2):113-8.

124. Buckley BS, Simpson CR, McLernon DJ, et al. Considerable differences exist between prevalent and incident myocardial infarction cohorts derived from the same population. *J Clin Epidemiol* 2010;63(12):1351-7.

125. Trivedi DP, Doll R, Khaw KT. Effect of four monthly oral vitamin D3 (cholecalciferol) supplementation on fractures and mortality in men and women living in the community: randomised double blind controlled trial. *BMJ* 2003;326(7387):469.

126. Prince RL, Austin N, Devine A, et al. Effects of ergocalciferol added to calcium on the risk of falls in elderly high-risk women. *Arch Intern Med* 2008;168(1):103-8.

127. Brazier M, Grados F, Kamel S, et al. Clinical and laboratory safety of one year's use of a combination calcium + vitamin D tablet in ambulatory elderly women with vitamin D insufficiency: results of a multicenter, randomized, double-blind, placebo-controlled study. *Clin Ther* 2005;27(12):1885-93.

128. LaCroix AZ, Kotchen J, Anderson G, et al. Calcium plus vitamin D supplementation and mortality in postmenopausal women: the Women's Health Initiative calcium-vitamin D randomized controlled trial. *J Gerontol A Biol Sci Med Sci* 2009;64(5):559-67.

129. Hsia J, Heiss G, Ren H, et al. Calcium/vitamin D supplementation and cardiovascular events. *Circulation* 2007;115(7):846-54.

130. Wang TJ, Pencina MJ, Booth SL, et al. Vitamin D deficiency and risk of cardiovascular disease. *Circulation* 2008;117(4):503-11.

131. Kilkkinen A, Knekt P, Aro A, et al. Vitamin D status and the risk of cardiovascular disease death. *Am J Epidemiol* 2009;170(8):1032-9.

132. Moher D, Schulz KF, Altman D. The CONSORT statement: revised recommendations for improving the quality of reports of parallel-group randomized trials. *JAMA* 2001;285(15):1987-91.

133. von Elm E, Altman DG, Egger M, et al. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. *Lancet* 2007;370(9596):1453-7.

134. Giovannucci E, Liu Y, Hollis BW, et al. 25-hydroxyvitamin D and risk of myocardial infarction in men: a prospective study. *Arch Intern Med* 2008;168(11):1174-80.

135. Marniemi J, Alanen E, Impivaara O, et al. Dietary and serum vitamins and minerals as predictors of myocardial infarction and stroke in elderly subjects. *Nutr Metab Cardiovasc Dis* 2005;15(3):188-97.

136. Pittas AG, Lau J, Hu FB, et al. The role of vitamin D and calcium in type 2 diabetes. A systematic review and meta-analysis. *J Clin Endocrinol Metab* 2007;92(6):2017-29.

137. Avenell A, Cook JA, MacLennan GS, et al. Vitamin D supplementation and type 2 diabetes: a substudy of a randomised placebo-controlled trial in older people (RECORD trial, ISRCTN 51647438). *Age Ageing* 2009;38(5):606-9.

138. Liu J, Tan H, Jeynes B. Serum 25OH vitamin D level, femur length, and risk of type 2 diabetes among adults. *Appl Physiol Nutr Metab* 2011;36(2):264-70.

139. Pittas AG, Dawson-Hughes B, Li T, et al. Vitamin D and calcium intake in relation to type 2 diabetes in women. *Diabetes Care* 2006;29(3):650-6.

140. Shankar A, Sabanayagam C, Kalidindi S. Serum 25-hydroxyvitamin d levels and prediabetes among subjects free of diabetes. *Diabetes Care* 2011;34(5):1114-9.

141. 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):847-55.

142. Forman JP, Curhan GC, Taylor EN. Plasma 25-hydroxyvitamin D levels and risk of incident hypertension among young women. *Hypertension* 2008;52(5):828-32.

143. Forman JP, Giovannucci E, Holmes MD, et al. Plasma 25-hydroxyvitamin D levels and risk of incident hypertension. *Hypertension* 2007;49(5):1063-9.

144. Snijder MB, Lips P, Seidell JC, et al. Vitamin D status and parathyroid hormone levels in relation to blood pressure: a population-based study in older men and women. *J Intern Med* 2007;261(6):558-65.

145. Rossouw JE, Finnegan LP, Harlan WR, et al. The evolution of the Women's Health Initiative: perspectives from the NIH. *J Am Med Womens Assoc* 1995;50(2):50-5.

146. Design of the Women's Health Initiative clinical trial and observational study. The Women's Health Initiative Study Group. *Control Clin Trials* 1998;19(1):61-109.

147. Anderson GL, Manson J, Wallace R, et al. Implementation of the Women's Health Initiative study design. *Ann Epidemiol* 2003;13(9 Suppl):S5-17.

148. Ritenbaugh C, Patterson RE, Chlebowski RT, et al. The Women's Health Initiative Dietary Modification trial: overview and baseline characteristics of participants. *Ann Epidemiol* 2003;13(9 Suppl):S87-97.

149. 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):321-33.

150. Health risks outweigh benefits for combined estrogen plus progestin. Clinical trial stopped early in major study. *Ginecol Obstet Mex* 2002;70:411-2.

151. Stefanick ML, Cochrane BB, Hsia J, et al. The Women's Health Initiative postmenopausal hormone trials: overview and baseline characteristics of participants. *Ann Epidemiol* 2003;13(9 Suppl):S78-86.

152. Anderson GL, Limacher M, Assaf AR, et al. Effects of conjugated equine estrogen in postmenopausal women with hysterectomy: the Women's Health Initiative randomized controlled trial. *JAMA* 2004;291(14):1701-12.

153. Jackson RD, LaCroix AZ, Cauley JA, et al. The Women's Health Initiative calcium-vitamin D trial: overview and baseline characteristics of participants. *Ann Epidemiol* 2003;13(9 Suppl):S98-106.

154. Standing Committee on the Scientific Evaluation of Dietary Reference Intakes FaNB. Dietary reference intakes for calcium, phosphorus, magnesium, vitamin D and fluoride. Washington, DC, 1999.

155. Moher D, Schulz KF, Altman DG. The CONSORT statement: revised recommendations for improving the quality of reports of parallel-group randomised trials. *Lancet* 2001;357(9263):1191-4.

156. Hays J, Hunt JR, Hubbell FA, et al. The Women's Health Initiative recruitment methods and results. *Ann Epidemiol* 2003;13(9 Suppl):S18-77.

157. WHI. Women's Health Initiative Study MANUALS: VOLUME 2 – PROCEDURES. (https://cleo.whi.org/studydoc/WHI%20and%20ES1%20Manual%20of%20Operations/1993-2005%20WHI%20CT%20and%20OS/Vol%202,%2007%20-%20Calcium%20and%20Vitamin%20D%20Intervenion%20(CaD).pdf). (Accessed 2/12/2013 2013).

158. Bibbins-Domingo K, Lin F, Vittinghoff E, et al. Predictors of heart failure among women with coronary disease. *Circulation* 2004;110(11):1424-30.

159. Wilhelmsen L, Rosengren A, Eriksson H, et al. Heart failure in the general population of men--morbidity, risk factors and prognosis. *J Intern Med* 2001;249(3):253-61.

160. He J, Ogden LG, Bazzano LA, et al. Risk factors for congestive heart failure in US men and women: NHANES I epidemiologic follow-up study. *Arch Intern Med* 2001;161(7):996-1002.

161. Chen YT, Vaccarino V, Williams CS, et al. Risk factors for heart failure in the elderly: a prospective community-based study. *Am J Med* 1999;106(6):605-12.

162. Eriksson H, Svardsudd K, Larsson B, et al. Risk factors for heart failure in the general population: the study of men born in 1913. *Eur Heart J* 1989;10(7):647-56.

163. Levy D, Larson MG, Vasan RS, et al. The progression from hypertension to congestive heart failure. *JAMA* 1996;275(20):1557-62.

164. Dunlay SM, Weston SA, Jacobsen SJ, et al. Risk factors for heart failure: a population-based case-control study. *Am J Med* 2009;122(11):1023-8.

165. Mosterd A, Hoes AW, de Bruyne MC, et al. Prevalence of heart failure and left ventricular dysfunction in the general population; The Rotterdam Study. *Eur Heart J* 1999;20(6):447-55.

166. Kalogeropoulos A, Georgiopoulou V, Kritchevsky SB, et al. Epidemiology of incident heart failure in a contemporary elderly cohort: the health, aging, and body composition study. *Arch Intern Med* 2009;169(7):708-15.

167. Levy BI. The mechanical properties of the arterial wall in hypertension. *Prostaglandins Leukot Essent Fatty Acids* 1996;54(1):39-43.

168. Lagakos SW. The challenge of subgroup analyses--reporting without distorting. *N Engl J Med* 2006;354(16):1667-9.

169. Rothwell PM. Treating individuals 2. Subgroup analysis in randomised controlled trials: importance, indications, and interpretation. *Lancet* 2005;365(9454):176-86.

170. Rothwell PM, Mehta Z, Howard SC, et al. Treating individuals 3: from subgroups to individuals: general principles and the example of carotid endarterectomy. *Lancet* 2005;365(9455):256-65.

171. Butler J, Kalogeropoulos A, Georgiopoulou V, et al. Incident heart failure prediction in the elderly: the health ABC heart failure score. *Circ Heart Fail* 2008;1(2):125-33.

172. Garland CF, Garland FC. Do sunlight and vitamin D reduce the likelihood of colon cancer? *Int J Epidemiol* 1980;9(3):227-31.

173. Dupont WD, Plummer WD, Jr. Power and sample size calculations. A review and computer program. *Control Clin Trials* 1990;11(2):116-28.

174. Adminstration FaD. Guideline for the format and content of the clinical and statistical sections of new drug applications. Rockville.

175. FISHER LD, DIXON, D.O., HERSON, J., FRANKOWSKI, R.K., HEARRON, M.S. and PEACE, K.E. . Intention to treat in clinical trials. In: PEACE KE, ed. *In*

Statistical Issues in Drug Research and Development. New York: Marcel Dekker 1990:331-50.

176. Lewis JA, Machin D. Intention to treat--who should use ITT? *Br J Cancer* 1993;68(4):647-50.

177. Bellamy SL, Lin JY, Ten Have TR. An introduction to causal modeling in clinical trials. *Clin Trials* 2007;4(1):58-73.

178. Cox DR. Regression Models and Life-Tables. *J Roy Stat Soc B* 1972;34(2):187-191.

179. Schoenfeld D. Partial Residuals for the Proportional Hazards Regression-Model. *Biometrika* 1982;69(1):239-41.

180. Heinze MKaG. PSHREG: A SAS macro for proportional and nonproportional subdistribution hazards regression with competing risk data. Vienna: Medical University of Vienna, 2012.

181. Xu R, O'Quigley J. Estimating average regression effect under non-proportional hazards. *Biostatistics* 2000;1(4):423-39.

182. Robins JM, Finkelstein DM. Correcting for noncompliance and dependent censoring in an AIDS Clinical Trial with inverse probability of censoring weighted (IPCW) log-rank tests. *Biometrics* 2000;56(3):779-88.

183. Brunner R, Dunbar-Jacob J, Leboff MS, et al. Predictors of adherence in the Women's Health Initiative Calcium and Vitamin D Trial. *Behav Med* 2009;34(4):145-55.

184. Simpson EH. The Interpretation of Interactions in Contingency Tables. *Journal of the Royal Statistical Society Series B (Methodological)* 1951;13(2):238 - 41.

185. Smith ML, Goltz HH. What is hidden in my data? Practical strategies to reveal Yule-Simpson's paradox and strengthen research quality in health education research. *Health Promot Pract* 2012;13(5):637-41.

186. Bolland MJ, Grey A, Gamble GD, et al. Calcium and vitamin D supplements and health outcomes: a reanalysis of the Women's Health Initiative (WHI) limited-access data set. *Am J Clin Nutr* 2011;94(4):1144-9.

187. Heshmat R, Tabatabaei-Malazy O, Abbaszadeh-Ahranjani S, et al. Effect of vitamin D on insulin resistance and anthropometric parameters in Type 2 diabetes; a randomized double-blind clinical trial. *Daru* 2012;20(1):10.

188. Fadda GZ, Akmal M, Lipson LG, et al. Direct effect of parathyroid hormone on insulin secretion from pancreatic islets. *Am J Physiol* 1990;258(6 Pt 1):E975-84.

189. de Boer IH, Tinker LF, Connelly S, et al. Calcium plus vitamin D supplementation and the risk of incident diabetes in the Women's Health Initiative. *Diabetes Care* 2008;31(4):701-7.

190. Wang G, Zhang Z, Ayala C, et al. Costs of heart failure-related hospitalizations in patients aged 18 to 64 years. *Am J Manag Care* 2010;16(10):769-76.

191. Buckley LM, Hillner BE. A cost effectiveness analysis of calcium and vitamin D supplementation, etidronate, and alendronate in the prevention of vertebral fractures in women treated with glucocorticoids. *J Rheumatol* 2003;30(1):132-8.

APPENDIX

Table 1

Heart failure assessment criteria

Criterion	Major Parameters/characteristics of criterion
Framingham	• Paroxysmal nocturnal dyspnea • Neck vein distention • Rales • Radiographic cardiomegaly (increasing heart size on chest radiography) • Acute pulmonary edema • S3 gallop • Increased central venous pressure (>16 cm H2O at right atrium) • Hepatojugular reflux • Weight loss >4.5 kg in 5 days in response to treatment Minor criteria: • Bilateral ankle edema

	• Nocturnal cough • Dyspnea on ordinary exertion • Hepatomegaly • Pleural effusion • Decrease in vital capacity by one third from maximum recorded • Tachycardia (heart rate>120 beats/min.)

Assessment	Diagnosis of heart failure	
	Supports if present	Opposes if normal or absent
Compatible symptoms	++	++
Compatible signs	++	+
Cardiac dysfunction on echocardiography	+++	+++
Response of symptoms or signs to therapy	+++	++
ECG		
Normal		++
Abnormal	++	+
Dysrhythmia	+++	+
Laboratory		
Elevated BNP/NT-proBNP	+++	+
Low/normal BNP/NT-proBNP	+	+++
Hyponatraemia	+	+
Renal dysfunction	+	+
Mild elevations of troponin	+	+
Chest X-ray		
Pulmonary congestion	+++	+
Reduced exercise capacity	+++	++
Abnormal pulmonary function tests	+	+
Abnormal haemodynamics at rest	+++	++

Boston	*Category I: history* • Rest dyspnea • Orthopnea • Paroxysmal nocturnal dyspnea • Dyspnea while walking on level area • Dyspnea while climbing *Category II: physical examination* • Heart rate abnormality (1 point if 91 to 110 beats per minute; 2 points if more than 110 beats per minute) • Jugular venous elevation (2 points if greater than 6 cm H_2O; 3 points if greater than 6 cm H_2O plus hepatomegaly or edema) • Lung crackles (1 point if basilar; 2 points if more than basilar) • Wheezing • Third heart sound *Category III: chest radiography* • Alveolar pulmonary edema • Interstitial pulmonary edema • Bilateral pleural effusion • Cardiothoracic ratio greater than 0.50 • Upper zone flow redistribution

WHI form for ascertaining heart failure – Form 121, page 4 of 7

Yes ☐1 No ☐0 **6. Congestive heart failure requiring and/or occurring during hospitalization.** (Physician diagnosis of new-onset or worsened congestive heart failure on this admission.)

6.1. Date of Admission ☐☐ - ☐☐ - ☐☐ (M/D/Y)

6.2. Congestive heart failure based on one or more of the following: ***(Mark all that apply.)***

☐1 Congestive failure diagnosed by physician and receiving medical treatment for CHF on this admission (e.g., diuretic, digitalis, vasodilator and/or angiotensin-converting enzyme inhibitor)

☐2 Congestive failure diagnosed by physician and receiving medical treatment on this admission **plus** current medical record documents a history of an imaging procedure showing impaired systolic or diastolic LV function

☐3 Pulmonary edema\congestion by chest X-ray on this admission

☐4 On this admission, dilated ventricle or poor left (or right-side) ventricular function (e.g., wall motion abnormalities) by echocardiography; radionuclide ventriculogram (RVG)/multigated acquisition (MUGA), or other contrast ventriculography, or evidence of left ventricular diastolic dysfunction

6.3. Was the congestive heart failure fatal? (Mark one.)

☐0 No, non-fatal

☐1 Yes, fatal ***(Complete Question 4 on page 3 of this form and Form 124 - Final Report of Death.)***

Table 2

Interaction between intervention and use of cardiovascular medication

Medication	P-value for interaction between intervention and use of cardiovascular medication		
	Overall cohort	Free of CV risk factors	Preexisting CV risk factors
ACE inhibitors	0.18	0.05	0.98
Betablockers	0.83	0.55	0.78
Antilipids	0.34	0.88	0.11
Calcium blockers	0.26	0.40	0.09
Composite	0.20	0.14	0.74

CV: cardiovascular

CURRICULLUM VITAE

MACARIUS M. DONNEYONG, MPH

Address **201 Abraham Flexner Way, Suite 1200, Louisville, KY 40202**
Phone**208-346-1099**
Email**mmdonn02@louisville.edu**

Education:

2009 –Present: Doctor of Philosophy, Epidemiology. University of Louisville, Louisville, KY.

Advisor: Carlton Hornung, PhD. Dissertation: "Vitamin D plus calcium supplementation among postmenopausal women: effect on risk of heart failure in the Women's Health Initiative study."

2007 – 2009: Master of Public Health, Epidemiology. Missouri State University, Springfield, MO.

2001 – 2005: Bachelor of Science, Nutrition and Food Science. University of Ghana, Accra, Ghana.

Professional Experience:

2012 – Present: Senior Research Associate. Division of Thoracic and Cardiovascular Surgery, University of Louisville School of Medicine, University of Louisville, Louisville, KY.

2009 – 2012: Graduate Research Assistant. Department of Epidemiology and Population Health, University of Louisville, Louisville, KY.

Summer 2009: Epidemiology Intern. Taney County Health Department, Branson, MO.

2008 – 2009: Graduate Assistant. Adult Diabetes Education Program, Missouri Area Health Education Center, Springfield, MO.

2007 – 2008: Graduate Research Assistant. Office of Student Success, Missouri State University, Springfield, MO.

2006 – 2007: Field Research Supervisor. Enhancing Child Nutrition through Animal Source Food Management (ENAM) Project, Iowa State University/University of Ghana, Ghana.

2006: Field Data Collector. Ghana Sustainable Change Project, AED(American Educational Development). Ghana.

2005 – 2006: Research Assistant. School of Public Health, University of Ghana, Accra, Ghana.

Awards:

2012: Scholarship for Ethics in Epidemiology workshop, American College of Epidemiology Conference. Chicago, IL.

2012: Summer Institute for Statistical Genetics Tuition and Travel Scholarship. Seattle, WA.

2012: Scholarship for Clinical and Translational Research Review Course. University of Louisville, Louisville, KY.

2012: Dissertation completion award. University of Louisville, Louisville, KY.

2010: Second place winner for poster presentation at Research Louisville. Louisville, KY.

2010: Missouri Outreach Graduate Opportunity (MOGO) Scholarship. Missouri State University, Springfield, MO.

Invited Scientific Presentations:

Sept. 2012: Poster Presentation. American College of Epidemiology Conference. Chicago, IL.

June 2011: Poster Presentation. 3rd North American Congress of Epidemiology. Montreal, Quebec, Canada.

Oct. 2010: Poster Presentation. Research Louisville. University of Louisville, Louisville, KY.

Sept. 2010: Poster Presentation. American College of Epidemiology Conference. San Francisco, CA.

April 2008. Oral Presentation. Inter-departmental Research Forum. Missouri State University, Springfield, MO.

Oct. 2006. Oral Presentation. The 6th Annual Research Meeting, Noguchi Memorial Institute For Medical Research. University of Ghana, Accra, Ghana.

June 2006. Poster Presentation. Africa Nutritional Epidemiology Conference II. Accra, Ghana.

Bibliography:

Peer-reviewed Articles/Abstracts:

1. **Donneyong, MM**, et. al. Outdoor Leisure-Time Physical Activity, Serum Vitamin D and Their Effects on CVD Mortality Risk. Abstracts of American College of Epidemiology Conference, September 8 – 11, 2012 Chicago, IL. Annals of Epidemiology Vol. 22, Issue 9, Page 666.

2. **Donneyong, MM** and Hornung, CA. Quality of life among congestive heart failure patients: A structural equation model. Abstracts of the 3rd North American Congress of Epidemiology, June 21-24, 2011 Montreal, Canada. American Journal of Epidemiology Volume 173 suppl 11.

3. Peiper, NC, **Donneyong, MM**, Hornung, CA. Quality of life outcomes among heart failure patients who smoke. Abstracts of the 3rd North American Congress of Epidemiology, June 21-24, 2011 Montreal, Canada. American Journal of Epidemiology Volume 173 suppl 11.

4. **Donneyong, MM** and Hornung, CA. Quality of life among congestive heart failure patients. Abstracts of American College of Epidemiology Conference, September 8 – 11, 2010 San Francisco, CA. Annals of Epidemiology Vol. 20, Issue 9, Page 704.

5. Kira C. Taylor, Digna Edwards, Todd Edwards, Daniel S. Evans, Guo Li, Kari E. North, Nora Franceschini, Rebecca Jackson, **Macarius Donneyong**, Andrea Z. LaCroix, John A. Robbins, Beth Lewis, Marcia L. Stefanick, Yongmei Liu, Melissa Garcia, and Jane A. Cauley. Genome-Wide Association Scan of Osteoporotic Fracture: A Meta-Analysis of 10,014 African-American Women. Abstracs of American Society of Human Genetics Annual Conference, Nov. 4-8 2012, San Francisco, CA.

6. **Macarius M. Donneyong**, Jaimin Trivedi, Mark S. Slaughter. Association of Prior Implantation of HeartMate II Left Ventricular Assistive Device with Post-

Heart Transplant Mortality. Abstract accepted for oral presentation at 33rd Annual Meeting and Scientific Sessions of the International Society for Heart and Lung Transplant, April 24-27, 2013, Montréal, Canada.

Teaching and Invited Lectures:

Guest Speaker. "Application of the CONSORT (Consolidated Standards of Reporting Trials) checklist". Clinical and Translational Research (1 credit, graduate students). Instructor: Carlton A. Hornung. University of Louisville, Louisville, KY. Summer 2012.

Teaching Assistant. Genetic and Molecular Epidemiology of Cardiovascular Diseases (3 credits, graduate students). Instructor: Kira Taylor, PhD. Department of Epidemiology, University of Louisville, Louisville, KY. Spring 2012.

Student Tutor. Physics 101 and Introductory Mathematics (Undergraduate students). RESOURCES FOR ACADEMIC ACHIEVEMENT (REACH) Program. University of Louisville, Louisville, KY. Fall 2009.

Instructor. Physics, Mathematics, and Integrated Science (High School students). Nandom Senior High School, Nandom, Ghana. 2001, and summers of 2003 – 2005.

Skills

Research/Data management

- Experienced in working with large research databases such as NHANES, WHI, UNOS, etc.
- Have diverse analytical skills and experience that span both the health and social sciences

Statistical software

- Proficient in SAS, PASW, AMOS
- Experience with R, HaploView, PLINK, METAL, QTL Cartographer, WEKA, TreeAge

Additional Training:

Clinical and Translational Research Review Course. University of Louisville, Louisville, KY. August 6 – 28, 2012.

Statistical Genetics Course. 17th Summer Institute in Statistical Genetics. Seattle, WA. July 17 – 27, 2012.

National Heart, Lungs, and Blood Institute (NHLBI)-North Western University Populations Workshop. Chicago, IL. July 11 – 14, 2010.

Membership:

American College of Epidemiology (ACE)

- Publication Committee
- Minority Affairs Committee