LSAW Vascular

Description	Variable	1998		1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2010	2013
		B/L	4 mo	12 mo	24 mo	36 mo	48 mo	60 mo	72 mo	84 mo	96 mo	114 mo	120 mo	144 mo	188 mo
Diary Returns	**Medications record - see medication data dictionary**	1500		1375				1136		1012			829		495
	Adverse event recording - see adverse event data dictionary	1500		1364	1334	1297	1198	1161	1102	1031	977	990	889	657	495
Prevalent and incident disease	**hospital admission discharge coding-atherosclerotic vascular disease, cardiovascular disease**														Complete 1998-2013*
Risk Factors	**Body mass index**	1498		1374	1317	1276	1206	1167		989			821	588	231
	Systolic, diastolic and mean arterial blood pressure	1450													
	Prescription of anti-hypertensive medications	1500						1161							
	Lipid profiles, Cholesterol, Triglycerides, LDLC HDLC	1032													
	Diabetes	1500													

	Smoking history	1500													
Risk prediction	**Framingham risk scores (BMI)**	1448													
	Framingham risk score (lipids)	1038													
Subclinical atherosclerosis	**Common Carotid Ultrasound (IMT and CCA)**					1136									
	Lateral spine DXA abdominal aortic calcification score (AAC8 and 24)	212		874				1073							
Vascular biomarkers	**High sensitivity CRP**	100													390
	Serum Cr, Albumin, Ca and Phos	1333	110	110	107	105		1125					770		392
	Serum cardiac troponin I	1235													
	Serum NGAL	1245													
	Serum K, Na, Cl, Bicarbonate, protein, anion gap	250													
	Brain natriuretic peptide	1023													
	Red cell folate			1214											
	Homocysteine			1214											
	Glycated hemoglobin (HbA1c)			1290											392
	Full Blood Cell Count	118													

	Osteoprotegerin	1333													
	RANKL	403													
	WNT/Frizzled antagonists (WIF-1, SFRP3 and DKK1)	1,109													
	25 OHD3 LC-MS/MS	1383					78								
	25 OHD2 LC-MS/MS	1383													
	Heavy Metals	1359													

* For “Complete 1998-2013” variables please contact admin@lsaw.com.au for further information

Overview

Participants

The participants involved in this study were recruited in 1998 to a 5-year, randomized, controlled trial of oral calcium supplements to prevent osteoporotic fractures as described previously [1]. Briefly, women were recruited from the Western Australian general population of women aged over 70 years by mail using the electoral roll a requirement of citizenship. Over 99% of Australians of this age are registered on the roll. Of the 5,586 women who responded to a letter inviting participation 1,510 women were willing and eligible and of these 1,460 women were recruited for the study. Participants were ambulant and did not have any medical conditions likely to influence 5-year survival. They were excluded if they were receiving bone-active agent, including hormone replacement therapy. Participants were similar in terms of disease burden and pharmaceutical consumption to whole populations of this age but they were more likely to be from higher socio-economic groups [2]. In the 5 years of the trial, participants received 1.2 g of elemental calcium as calcium carbonate daily or a matched placebo.

Overview of CAIFOS randomized controlled trial

Patients received calcium carbonate tablets, 0.6g twice per day (with morning and evening meals), or identical placebo tablets (Wyeth Consumer Healthcare, Baulkham Hills, Australia). The randomization list was produced by generating 146 blocks of 10 numbers. In each block, 5 positions representing placebo and 5 positions representing calcium treatment were ordered using a letter code according to a random number generator. The numbered blocks were ordered according to randomly generated numbers, and an identification number was assigned in order to each letter code in the randomized list. The Pharmacy Department of the Sir Charles Gairdner Hospital, Nedlands, Australia, assigned a treatment to the letter code and assigned the appropriate medications to the patient according to this list. The randomization was stratified by allocating patients to blocks according to whether a prevalent non-traumatic fracture had occurred after age 50 years, ensuring that an equal number of patients with and without a prevalent fracture received placebo or calcium. Medication compliance was checked at the completion of the study by counting returned tablets at each 12-month review and was calculated as a percentage of the optimum. Average yearly compliance of less than 80% was classified as non-compliant.

Ethics statement

The Human Ethics Committee of the University of Western Australia approved the study and written informed consents were obtained from all participants.

Medications use – verified with GP where possible

The participants provided their previous medical history and current medications verified by their General Practitioner. These data were coded using the International Classification of Primary Care – Plus (ICPC-Plus) method [3]. The coding methodology allows aggregation of different terms for similar pathologic entities as defined by the ICD-10 coding system. These data were then used to determine the presence of pre-existing diabetes (T89001-90009). Cardiovascular medications included beta-blockers, angiotensin-converting enzyme inhibitors, angiotensin II receptor blockers, HMG-Co A reductase inhibitors and anti-platelet agents. Participants' medical histories and medications were verified by their General Practitioners where possible.

Prevalent Disease status

Prevalent Atherosclerotic vascular disease, ischemic heart disease, cerebrovascular disease, heart failure and peripheral arterial disease

Atherosclerotic hospitalizations were retrieved from the Western Australian Data Linkage System (WADLS) for each of the study participants from 1980-1998. WADLS provides a complete validated record of every participant's hospitalizations from the coded records of each participant's hospital admissions. Atherosclerotic events were defined using the principal discharge diagnosis codes from the International Classification of Diseases, Injuries and Causes of Death Clinical Modification (ICD-9-CM) [4]. These codes included: ischemic heart disease (ICD-9-CM codes 410-414); heart failure (ICD-9-CM code 428); cerebrovascular disease excluding haemorrhage (ICD-9-CM codes 433-438); and peripheral arterial disease (ICD-9-CM codes 440-444).

Data linkage

Nb: There have been three data extractions of the Hospital Data Morbidity Collection, Mortality Registry, Emergency Data Collection and Cancer Registry to date (2010, 2012 and 2014). The numbers may vary between data extraction as more sensitive and specific linkage methodologies have been implemented and therefore, we recommend using the most up to date data extraction available.

Death certificate cause of death (Primary cause of death with contributing causes of death)

Mortality was defined using code death certificate data from the International Classification of Diseases, Injuries and Causes of Death Clinical Modification (ICD-9-CM) [4] and the International Statistical Classification of Diseases and Related Health Problems, 10th Revision, Australian Modification (ICD-10- AM) [5]. These codes included: ICD-9-CM codes 390-459 and ICD-10-AM codes I00-I99. Cancer mortality records were retrieved from WADLS from the Western Australian Cancer registry. The search for ICD codes included all available diagnostic information that comprised Parts 1 and 2 of the death certificate. All diagnosis text fields from the death certificate were used to ascertain the cause(s) of deaths where these coded data were not yet available from the WADLS.

Atherosclerotic vascular disease, cardiovascular disease

The primary outcome was an atherosclerotic vascular disease event causing hospitalization or death. First-time atherosclerotic hospitalizations were retrieved from the Western Australian Data Linkage System (WADLS) for each of the study participants from 1998 until 10 years after their baseline visit. WADLS provides a complete validated record of every participant's primary diagnosis at hospital discharge using coded data form all hospitals in Western Australia. Cause of death was retrieved from the coded death certificate using information in Parts 1 and 2 of the death certificate. Atherosclerotic events were defined using primary diagnosis codes from the International Classification of Diseases, Injuries and Causes of Death Clinical Modification (ICD-9-CM) [4] and the International Statistical Classification of Diseases and Related Health Problems, 10th Revision, Australian Modification (ICD-10- AM) [5]. These codes included: ischemic heart disease (ICD-9-CM codes 410-414 and ICD-10-AM codes I20-I25); heart failure (ICD-9-CM code 428 and ICD-10-AM code I50); cerebrovascular disease excluding hemorrhage (ICD-9-CM codes 433-438 and ICD-10-AM codes I63-69, G45.9); and peripheral arterial disease (ICD-9-CM codes 440-444 and ICD-10-AM codes I70-74).

Anthropometric and body composition assessments

Body weight (kg) was assessed using digital scales with participants wearing light clothes and no shoes. Height (cm) was assessed by using a stadiometer, and body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters. Body composition was measured in a randomly selected subgroups of participants by whole body Dual energy X-ray absorptiometry (DXA), using a Hologic 4500A bone densitometer (Hologic Corp., Waltham, MA) with CVs under 2% in our laboratory. All data and analyses presented exclude the head. Lean body mass refers to bone-free lean mass. The lean mass of the arms and legs were summed to provide the appendicular skeletal muscle mass.

Blood pressure

Blood pressure was measured on the right arm with a mercury column manometer using an adult cuff after the participants have been seated in an upright position and had rested for 5 minutes. An average of three blood pressure readings was recorded.

Smoking history

Smoking status was coded as non-smoker or ex-smoker/current smoker if they had consumed more than 1 cigarette per day for more than 3 months at any time in their life.

Physical activity

Physical activity was assessed by a demographic questionnaire where participants reported their type of activity and hours per week[6] in the question "Please list any sports recreation or regular physical activity, including walking, that you undertook in the last three months". The energy cost of such activities is given in METs[7] (1 MET accounts for an individual's basal metabolic rate and equals ~ 1kcal/kg/h). Activity levels (kcal/day) were calculated multiplying frequency, duration, energy cost of the activities and the body weight of individuals.

Carotid - intima medial thickness

A pre-planned ancillary study at year 3 to investigate epidemiological determinants of CCA-IMT and carotid atherosclerosis by B-mode carotid ultrasound examination was undertaken in 1,183 participants of the original cohort. Informed consent was obtained and the Human Ethics Committee of the University of Western Australia approved the study. The presence of carotid focal plaques and common carotid artery intimal medial thickness (CCA-IMT) were determined at year 3. Assessment were performed using B-mode carotid ultrasound examination by the same sonographer with a 8.0 mHz linear array transducer fitted to an Acuson Sequoia 512 ultrasound machine using a standard image acquisition protocol in 2001 [8]. The far walls of the distal 2cm of the left and right common carotid arteries were examined and images were taken from 3 different angles (anterolateral, lateral and posterolateral) to account for the possibility of asymmetrical wall thickening. End-diastolic images were recorded and a semi-automated edge-detection software program was used for image analysis. The same technician performed off-line analysis of all of the images. After assessment of CCA-IMT and focal plaque on the right side, the process was repeated on the left side. The CCA-IMT from each of the 6 images (3 on either side) was averaged to give an overall mean CCA-IMT. Once IMT images were recorded, the entire carotid tree (CCA, carotid bulb, internal and external carotid) was examined for the presence of focal plaque defined as a clearly identified area of focal increased thickness (≥ 1 mm) of the intima-media layer. A short-term precision study of 20 non-trial subjects with repeat IMT measurements between 0 and 31 days apart (mean 10.3 days) was performed which yielded a CV of 5.98% as described previously [9].

Framingham risk score (FRS) scores

The Framingham risk scores were calculated based on the publication by D'Agostino et al [10] and were then confirmed using the online calculator prepared by R.B. D'Agostino and M.J. Pencina at [https://www.framinghamheartstudy.org/risk-functions/cardiovascular-disease/10-year-risk.php#].

Abdominal aortic calcification scores (AAC8 and 24) from lateral spine DXA

All abdominal aortic calcification scores from 0 to 24 or 0 to 8 were derived from digitally enhanced lateral single-energy images of the thoraco-lumbar spine using a Hologic 4500A machine (Hologic, Bedford, MA, USA). A single experienced investigator (John T Schousboe) read all images using the established technique [11-13] blinded to carotid artery measures of atherosclerosis. For AAC 24 the severity of AAC was categorized using previously published groupings: low (AAC24 score 0 or 1), moderate (AAC24 score 2-5) and severe (AAC24 score 6 or greater) AAC scores [13].

High sensitivity C reactive protein

The hs-CRP assay was performed on a Hitachi 917 analyser using the Roche hs-CRP assay. It uses a particle enhanced immunoassay system with an assay range from 0.1 to 20 mg/L. For values > 1mg/L the CV of the assay is <5%.

Serum Cr, Albumin, Ca and Phosphate
Blood samples were analysed for alkaline phosphatase (ALP), creatinine, calcium, and phosphorus, using routine methods (BM/Hitachi 747 Analyser, Boehringer Mannheim GmbH, Mannheim Germany).

Lipid profiles
Total cholesterol, high-density lipoprotein cholesterol and triglyceride concentrations were determined using a Hitachi 917 auto analyser (Roche diagnostics). Low-density lipoprotein cholesterol was calculated using Friedewald's method [14].

High sensitivity cardiac troponin I (hs-TnI)
High sensitivity cardiac troponin I was measured in 2013 in baseline serum from 1,235 samples stored at -80C using the Abbott ARCHITECT i2000SR STAT hs-TnI assay. The LoD for the assay is 1.9 pg/mL however observed values below this limit (n=5, range 1.4-1.9 pg/mL) were included [assay range 0-50,000 pg/mL].

Serum neutrophil gelatinase-associated lipocalin (NGAL or lipocalin 2)
Plasma NGAL was measured using a two-step chemiluminescent microparticle monoclonal immunoassay on an automated platform (Abbott Diagnostics, Longford, Ireland). The inter-assay coefficient variation over a 3-week period was 6.07% and 2.63% at concentrations of 21 and 1,194 ng/L respectively.

Brain natriuretic peptide (BNP)
Plasma samples were stored at -80^0C, only samples that had not been previously defrosted were selected for analysis due to the BNP molecules stability. Once defrosted, thawed samples were mixed thoroughly by inversion and centrifuged for 10 minutes at 4,000 rounds per minute (rpm) with 400µL aliquotted into the 1mL Architect cups. Aliquots were loaded onto the Architect (Abbott, Abbott Park, Illinois, USA) at *PathWest* (Nedlands, WA) for analysis according to the BNP assay protocol. The BNP assay measurement range was < 10 to >500pg/mL.

Red cell folate
Red blood cell folate levels were determined using a chemiluminescence method on an ACS:180 Immunoassay analyser (Chiron Diagnostics Corporation/Bayer, CA). The inter-assay CV for red cell folate measurement was less than 12%.

Homocysteine
The plasma total L-homocysteine (tHcy) was measured using a Fluorescence Polarization Immuno-assay on an Abbott IMx Analyzer (Abbott Laboratories, Abbott Park, IL). The inter-assay CV for homocysteine measurement was less than 5%. The tHcy includes free monomeric homocysteine, free dimeric homocysteine, protein bound forms and mixed dimeric low molecular mass forms.

Glycated hemoglobin (HbA1c)
In 1999 glycated hemoglobin was measured using Ion-exchange HPLC using the Variant II (Bio-Rad), the CV was 2.00% at 5.4 and 1.44% at 13.7. Additionally fasting blood samples were collected at baseline (1998).

Osteoprotegerin (OPG) and RANKL
Free OPG was measured in 2005 using the baseline sera from 1,333 (89%) participants using a validated enzyme immunoassay (R&D Systems, Minneapolis, MN, USA) as previously described [15,16]. The reported intra- and inter-assay coefficients of variation of the immunoassays were 3.6% and 10.6% respectively [16]. Serum levels of and RANKL were quantified by EIA (Biomedica GmBH, Austria). Serum osteocalcin was measured using radioimmunoassay techniques as previously described[15].

WNT/Frizzled antagonists (WIF-1, SFRP3 and DKK1)
Serum Dickkopf1 (Dkk1), secreted Frizzled-related protein 3 (sFRP3) and Wnt inhibitory factor 1 (WIF1) levels were assessed in 1,112 participants had Dkk-1 levels were determined in the year 2013 using enzyme immunoassay (EIA) provided by R&D Systems (Minneapolis, MN). The inter- and intra-assay coefficients of variation (CV) was <10% for these assays.

Circulating 25(OHD)3 and 25(OHD)2 by LC-MS/MS
Fasting blood samples were collected at baseline (1998). Plasma $25OHD_2$ and $25OHD_3$ concentrations were determined using a LC-MS/MS (Liquid Chromatography Tandem Mass Spectrometry) method at the RDDT Laboratories (Bundoora, VIC, Australia) according to published methodology [17]. Between-run coefficients of variation (CVs) were 10.1% at a $25OHD_2$ mean concentration of 12 nmol/L and 11.3% at a $25OHD_3$ mean concentration of 60 nmol/L. Seasonally adjusted values were calculated using the methods described by others

1. Prince RL, Devine A, Dhaliwal SS, Dick IM. Effects of calcium supplementation on clinical fracture and bone structure: results of a 5-year, double-blind, placebo-controlled trial in elderly women. Arch Intern Med 2006;166:869-75.
2. Bruce DG, Devine A, Prince RL. Recreational physical activity levels in healthy older women: the importance of fear of falling. J Am Geriatr Soc 2002;50:84-9.
3. Britt H, Scahill S, Miller G. ICPC PLUS for community health? A feasibility study. Health Inf Manag 1997;27:171-5.
4. World Health Organization. Manual of the international statistical classification of diseases, injuries, and causes of death : based on the recommendations of the ninth revision conference, 1975, and adopted by the Twenty-ninth World Health Assembly. 1975 revision. ed. Geneva: World Health Organization; 1977.
5. World Health Organization. ICD-10 : international statistical classification of diseases and related health problems : tenth revision. 2nd ed. Geneva: World Health Organization; 2004.

6. Devine A, Dhaliwal SS, Dick IM, Bollerslev J, Prince RL. Physical activity and calcium consumption are important determinants of lower limb bone mass in older women. J Bone Miner Res 2004;19:1634-9.
7. WD M, FI K, VL K. Energy, Nutrition and Human Performance. Philadelphia, Pa: Lea & Febiger 1991.
8. Salonen JT, Salonen R. Ultrasound B-mode imaging in observational studies of atherosclerotic progression. Circulation 1993;87:II56-65.
9. Ivey KL, Lewis JR, Hodgson JM, et al. Association between yogurt, milk, and cheese consumption and common carotid artery intima-media thickness and cardiovascular disease risk factors in elderly women. Am J Clin Nutr 2011;94:234-9.
10. D'Agostino RB, Sr., Vasan RS, Pencina MJ, et al. General cardiovascular risk profile for use in primary care: the Framingham Heart Study. Circulation 2008;117:743-53.
11. Schousboe JT, Wilson KE, Kiel DP. Detection of abdominal aortic calcification with lateral spine imaging using DXA. J Clin Densitom 2006;9:302-8.
12. Schousboe JT, Wilson KE, Hangartner TN. Detection of aortic calcification during vertebral fracture assessment (VFA) compared to digital radiography. PLoS One 2007;2:e715.
13. Schousboe JT, Taylor BC, Kiel DP, Ensrud KE, Wilson KE, McCloskey EV. Abdominal aortic calcification detected on lateral spine images from a bone densitometer predicts incident myocardial infarction or stroke in older women. J Bone Miner Res 2008;23:409-16.
14. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 1972;18:499-502.
15. Ueland T, Bollerslev J, Wilson SG, et al. No associations between OPG gene polymorphisms or serum levels and measures of osteoporosis in elderly Australian women. Bone 2007;40:175-81.
16. Ueland T, Bollerslev J, Godang K, Muller F, Froland SS, Aukrust P. Increased serum osteoprotegerin in disorders characterized by persistent immune activation or glucocorticoid excess--possible role in bone homeostasis. Eur J Endocrinol 2001;145:685-90.
17. Maunsell Z, Wright DJ, Rainbow SJ. Routine isotope-dilution liquid chromatography-tandem mass spectrometry assay for simultaneous measurement of the 25-hydroxy metabolites of vitamins D2 and D3. Clinical chemistry 2005;51:1683-90.