LSAW Bone, Joint, Muscle and Falls

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	180 mo
Anthropometry	**Height and weight**	1495		1376	1317	1276	1159	1136		989			823	583	231
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
Hospital discharge data	**Hip and all fracture hospitalisation**														Complete 1998-2013
	Injurious fall hospitalisation														Complete 1998-2013
	Osteoarthritis and joint replacement hospitalisation														Complete 1998-2013
Bone structural variables															
Spine	**DXA Spine BMD**	244		243	108	102	101	1114		787					
	Kyphosis index	560						1265							

	MXA thoracic-lumbar spine	515		1140				1057							
	Spine fracture VFA report	212		874				415							
Hip	**DXA Total hip BMD**	531		1129	105	94	101	1101		778			599	376	221
	QCT hip											91			
	Hip structural analysis	1162		821											
Other skeletal areas	**DXA Whole body**	419		339	103	95	101	918		751			600	380	
	pQCT Arm 4%							754		786			500	310	
	pQCT Arm 15%							512		786			514	342	
	pQCT Tibia 4%							778		774			552	350	
	pQCT Tibia 15%									790			554	343	
	Calcanel ultrasound Heel BUA, SOS, Stiffness	1478			1318			1164		920			806	532	
Bone Biochemistry	**Total alkaline phosphatase**	1279	112	110	107	105		216							
	Osteocalcin	290	112	108	108	108									
	OPG	1333													
	RANKL	403													
	Deoxypyridinoline creatinine ratio	293	110	110	107	105		216							
	Parathyroid hormone	299	112	109	108	108		245							

	25 OHD LC MS/MS	1383													
	Calcitriol	120	109												
	DBP	120	109												
	Gut calcium absorption 10mg dose	119			105		119								
	Gut calcium absorption 600mg dose		113												
	Serum Cr, Albumin, Ca and Phos	1352	112	110	107	105		1125					770		
	Spot urine Ca, Phos, Cr	293	111	110	107	107		216							
	Anion Gap	254													
	Spot urine Na, Cl, K	292													
	24h urine N, K, Cr	343													
Sex homones	**Estradiol**	1479													
	Sex hormone binding globulin (SHBG)	1353	80												
	Follicle stimulating hormone	115	80			613									
	Luteinising hormone					617									

Muscle function balance and mobility	**Rhomberg -eyes open**	1488						1179		984			780	558	
	Rhomberg -eyes closed	1488						1177		981			774	556	
	Timed up and go test	1497						1178		982			797	566	
	Barthel score	1496						1212		1020			893	651	
	Grip strength	1488						1179		988			778	559	
	Chair rise power												438		
Other biochemical variables	**Red cell folate**			1214											
	Homocysteine			1214											
	Glycated Hb			1290											392
	ALT, GGT	293	112	112	107	105	105								

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. Human ethics approval for the use of linked data for the project was provided by the Human Research Ethics Committee of the Western Australian Department of Health (DOHWA HREC), project number #2009/24.

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.

DXA and Hip Structural Analysis

Femoral geometric indices were determined at three narrow 'cross-sectional' regions traversing the proximal femur, using the Hip Structural Analysis (HSA) Program Version 3 and incorporating correction for the array

mode of the QDR 4500.[4] The regions correspond to the "narrow neck", which traverses the femoral neck at its narrowest point, the "intertrochanteric region", which is positioned along the bisector of the angle between the neck and shaft axes, and the "shaft region", positioned at a distance of 1.5 times minimum neck width, distal to the axes intersection. The methods of computation and the derived variables have been described elsewhere.[5-8] All DXA scans were acquired using the same Hologic QDR 4500A scanner (Bedford MA, USA). As the primary outcome of the RCT was fracture, to reduce responder burden BMD data were not collected until the 12-month visit. At this time, 1390 volunteers remained in the study and these women were encouraged but not required to attend a second visit within one or two weeks of the primary follow-up visit for DXA scanning. A total of 1100 women had hip scans. Data from 43 of these women could not be utilised for HSA analysis. Scans were excluded when the program failed to complete a full analysis at all three regions (1.64%) or failed to correctly identify regions of interest (1.27%). Scans were also excluded where insufficient abduction during DXA scan acquisition resulted in the ischium being located too close to the femoral neck, so that overlap of the bones caused erroneous measurements (1.0%). The variables included were BMD measured at each HSA site, cross-sectional area (CSA), section modulus (Z), subperiosteal width (SPW) and the centroid position. Cross-sectional area is an index of the resistance of the bone to compression. It is equal to the amount of bone surface area in the cross-section after excluding all soft tissue space and is therefore proportional in principle to bone mineral content (BMC).[8] Higher section modulus (Z) indicates greater resistance of the bone to mechanical failure under bending.[4,8] It is derived from cross-sectional moment of inertia (CSMI), which is an important index of structural rigidity. Due to limitations of 2D DXA images the CSMI and Z are only relevant to bending in the plane of the DXA image (~the coronal plane) and cannot assess bending in any other direction.[4-6] The subperiosteal width (SPW) is the outer diameter of the bone which is computed as the blur-corrected width of the mass profile.[4,8] The centroid position is the measured position of the centre of mass of the cross-section with respect to the medial bone margin and is normalised by dividing this position by the subperiosteal width.[4] A lower centroid value in a bone subject to bending implies that there is an imbalance between bone mass in the superolateral zone compared with the inferomedial zone, in favour of the latter. This could occur, for example, as a result of thinning of superior cortex with aging, as has been observed in the upper femoral neck.[38] This imbalance may reduce the resistance of the bone to local compressive or buckling forces and enhance fracture risk.[38] The HSA program also generates estimates of mean cortical thickness and cortical stability (buckling ratio). However, since these require assumptions concerning cross-sectional shape and the ratio of trabecular to cortical bone mass at each measured site, they have not been included.[4,8,9] All scans were analysed by a single operator after extensive training. Prior to the commencement of this analysis, 30 randomly selected scans were analysed in random order on two separate occasions one day apart to determine intra-operator reliability. ICCs varied between regions but were >0.96. No systematic difference occurred between the two analyses for any variable. This subset of scans were reanalysed on four occasions during the course of the analysis to ensure that operator technique remained consistent. At each reanalysis repeated measures ANOVA models were constructed to test for systematic error. No significant difference was present between the serial measurements. The short-term precisional errors of HSA variables, expressed in CV%, have been determined for the Hologic QDR4500 in a subject sample similar to this study, from replicate hip DXA scans.[31] For CSA it ranges from 2.3 – 3%; for CSMI 3.2 – 4.6%; for Z 2.5 to 4.0%; for subperiosteal width 0.9 – 2.1%; and for centroid position the range is 1.1 – 2.1%.

Bone measurements

Due to restricted availability of human resources, total hip aBMD of subjects were measured by Dual Energy X-ray Absorptiometry (DXA) using the same Hologic Acclaim 4500A fan beam densitometer (Hologic Corp, Waltham, MA, USA). The CV at the total hip was 1.2% in our laboratory (10). The total hip BMD T-score was determined using the American third National Health and Nutrition Examination Survey (NHANES III) reference database (11). The World Health Organization defines low bone density as BMD T-score between -1 to -2.5 and osteoporosis as T-score -2.5 or below (12). Therefore, we categorized subjects with hip BMD

T-score above -1 as normal bone mineral density, below -1 as having low bone density and below -2.5 as having osteoporosis. Quantitative heel ultrasound (QUS) of the calcaneus of the left foot was measured in duplicate using a Lunar Achilles Ultrasound machine (Lunar Corp., Madison, WI, USA) at baseline. The average measurement of the Speed of Sound (SOS), Broadband Ultrasound Attenuation (BUA) and Stiffness index (SI) were determined. The coefficients of variation (CV) were 0.4 for SOS and 1.6% for BUA.

Timed Up and Go Test

The Timed Up and Go test in which the patient is timed while rising from a chair, walking 3 meters, turning, returning to sit on the chair was performed at baseline(13). Subjects were allowed to practise once then timed. The inter-observer CV error was 7% in our laboratory as assessed on a random sample of 20 subjects.

Self-reported fracture ascertainment

Prevalent fractures were determined at baseline by obtaining a fracture history from each subject that included age at the time of fracture, the site, and how the fracture was sustained. A prevalent fracture was included if the fracture occurred after the age of 50 years; occurred with minimal trauma as defined by falling from a height of one metre or less; and not of the face, skull, fingers, or toes. Incident atraumatic clinical fractures and atraumatic symptomatic vertebral fractures were re-coded in an adverse events diary which was collected every 4 months during the first 5 years and 6 months during the second 5 years. The diagnosis of clinical vertebral and non-vertebral fractures was confirmed by reference to radiographic records. As BMD measurement was performed at year one, fractures from year one to ten were regarded as incident fracture in the data analysis, whereas fractures during the first year were treated as prevalent fracture.

Hospital and mortality-derived fracture outcomes

Prevalent fractures from the age of 50 years were self-reported with hospital discharge records searched for any fracture-related admissions from 1980-1998. Codes used for identification include S02-S92 and T02. Fractures of the face (S02.2-S02.6), fingers (S62.5-S62.7), and toes (S92.4-S92.5) and fractures caused by motor vehicle injuries were excluded. Incident fracture-related hospitalizations and deaths over 15 years were tracked through the Western Australian Hospital Morbidity Data system, which is part of the Western Australian Data Linkage System. Fracture events were determined using the International Statistical Classification of Diseases and Related Health Problems, 10th Revision (as above). Major osteoporotic fractures included those of the hip, spine, humerus, and wrist, as defined by the WHO FRAX algorithm (14). In addition, incident hip fracture data were retrieved from the Western Australia Hospital Morbidity Data System (HMDS) for each of the study participants from 1998 when they entered the study until 10 years after their baseline visit. As the HMDS captures coded diagnosis data pertaining to all public and private inpatient contacts in Western Australia(15), it allows complete ascertainment of verified hip fracture independently of patient report with the associated problems such as loss to follow-up.

Kyphosis index

Thoracic curvature was measured using a flexicurve ruler (16). The subjects were asked to stand adopting their normal posture and the ruler was pressed against their back with the top end placed on the seventh cervical spine in the midline. The ruler was moulded into the shape of the subject's spine in the midline to the level of the lumbosacral joint. The flexicurve ruler was removed and the shape of the spine was then traced onto paper and analysed as shown in Figure 1. The kyphosis index (KI) was calculated as the ratio of B to E multiplied by 100. The larger the KI the more marked the kyphosis. The coefficient of variation for KI of 6.6%, as determined from duplicate measurements performed in 20 subjects by a group of trained assessors on the same day, was half that reported by others (17). The validity and reliability of the flexicurve ruler method of kyphosis assessment in osteoporotic subjects has been compared to roentgenographic assessment and found to have close agreement (18).

Morphometric spinal deformities

The reference data for the ascertainment of spinal deformities was determined from measurement of MXA data of 120 patients of the same age as the study patients and recruited from the same population. Single energy high-definition lateral MXA scans of vertebra T4-L4 were performed using a Hologic QDR 4500A. For each vertebra a single operator placed six reference markers at the corners and in the middle of the upper and lower surface of each vertebra. The height was measured as the distance between the markers at the edge of the superior and inferior endplates of that vertebra as described by the operator's manual. The mean coefficient of variation in MXA measurements of the anterior, central and posterior heights was determined for the single operator who undertook the assessment of all the MXA scans by undertaking point placement from L4 to T12 in duplicate measurements from 30 subjects. The mean coefficients of variation were 4.5% for posterior height measurements, 4.5% for central height measurements and 5.3% for anterior height measurements. As described by McCloskey et al. (19) the ratio of the anterior to posterior height, the central to posterior height and the posterior to the predicted posterior height was calculated for the reference data and expressed as the 5% trimmed mean and SD (Table 1). The predicted posterior height was calculated from the two adjacent vertebrae above and below the vertebra of interest, or for T4 the four vertebrae below and for L4 the four vertebrae above. Prevalent wedge vertebral deformities were defined as both an anterior/posterior height ratio and anterior/posterior predicted height ratio three SD below the normative mean anterior to posterior value at that level; central deformities as both a central/posterior height ratio and central/posterior predicted height ratio three SD below the normative value at that level and prevalent crush vertebral deformities were defined as both a posterior/predicted posterior height ratio three SD below the normative value at that level and anterior/posterior predicted height ratio three SD above the normative value at that level for that measurement in our reference population (19). Incident vertebral deformities over the subsequent four years were defined as a reduction in the posterior, mid or anterior vertebral height of 20% or more from baseline.

Physical activity

Physical activity was assessed by a demographic questionnaire where participants reported their type of activity and hours per week(20) 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(21) (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. Physical activity levels were ascertained by questionnaire at baseline.[22] Women were asked whether they participated in any sports, recreation or regular physical activity. Those who answered "no" to this question scored zero and those who answered "yes" were asked to list up to four sports, recreations or forms of regular physical activity, including walking, which were undertaken in the last three months. Energy expenditure (kcal/day) for these activities was calculated using published energy costs.[23,24].

Estimated fracture risk

FRAX US Caucasian and FRAX Australian fracture risk prediction was determined using each calculator on the FRAX website (www.shef.ac.uk/FRAX/) . Baseline data were used to calculate the estimated 10-year risk of fracture using the FRAX Australian and FRAX US Caucasian calculators with and without aBMD. Data used including: age, sex, weight (Kg), height(cm), fracture history: previous fracture, parental history of hip fracture, current smoking, glucocorticoid use, history of rheumatoid arthritis or secondary osteoporosis and intake of 3 or more units per day of alcohol. Data was input with and without femoral neck DXA BMD (g/cm2).

Lipocalin 2

Blood samples were collected at baseline for assessment. Plasma lipocalin 2 was measured using a two-step chemiluminescent microparticle monoclonal immunoassay on an automated platform (Abbott Diagnostics, Longford, Ireland). The inter-assay coefficient of variation over a 3-week period was 6.07% and 2.63% at concentrations of 21 and 1,194ng/L respectively.

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).

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 (25,26). The reported intra- and inter-assay coefficients of variation of the immunoassays were 3.6% and 10.6% respectively (26). Serum levels of and RANKL were quantified by EIA (Biomedica GmBH, Austria). Serum osteocalcin was measured using radioimmunoassay techniques as previously described(25).

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 (27). 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

Estradiol, SHBG and free oestradiol index

In all subjects, a fasting venous blood sample was collected. Serum sex hormone binding globulin was measured at baseline using an immunochemiluminometric assay (Imulite, Los Angeles, USA), the inter and intra assay coefficient of variation (CV) were 7.1% and 6.8% respectively at 24nmol/L. Serum estradiol was measured at baseline using a radioimmunoassay (RIA, Orion Diagnostica, Espoo, Finland) with an analytical sensitivity of 5pmol/L. We found the inter-assay CV to be 6.6% at a mean of 101 pmol/L and 7.2% at a mean of 48 pmol/L. The intra-assay CV was 5.1% at a mean of 103 pmol/L and 7.5% at a mean of 49 pmol/L. Free estradiol index (FEI) was calculated as the molar ratio of estradiol to SHBG multiplied by 1000.

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