Osteoporos Int (2009) 20:1241–1251
DOI 10.1007/s00198-008-0776-y

ORIGINAL ARTICLE

Effects of a multi-component exercise program and calcium–vitamin-D_3-fortified milk on bone mineral density in older men: a randomised controlled trial

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Received: 30 April 2008 / Accepted: 18 August 2008 / Published online: 28 October 2008

Abstract
Summary We examined the independent and combined effects of a multi-component exercise program and calcium–vitamin-D_3-fortified milk on bone mineral density (BMD) in older men. Exercise resulted in a 1.8% net gain in femoral neck BMD, but additional calcium–vitamin D_3 did not enhance the response in this group of older well-nourished men.
Introduction This 12-month randomised controlled trial assessed whether calcium–vitamin-D_3-fortified milk could enhance the effects of a multi-component exercise program on BMD in older men.
Methods Men (n=180) aged 50–79 years were randomised into: (1) exercise + fortified milk; (2) exercise; (3) fortified milk; or (4) controls. Exercise consisted of high intensity progressive resistance training with weight-bearing impact exercise. Men assigned to fortified milk consumed 400 mL/day of low fat milk providing an additional 1,000 mg/day calcium and 800 IU/day vitamin D_3. Femoral neck (FN), total hip, lumbar spine and trochanter BMD and body composition (DXA), muscle strength 25-hydroxyvitamin D and parathyroid hormone (PTH) were assessed.
Results There were no exercise-by-fortified milk interactions at any skeletal site. Exercise resulted in a 1.8% net gain in FN BMD relative to no-exercise ($p<0.001$); lean mass (0.6 kg, $p<0.05$) and muscle strength (20–52%, $p<0.001$) also increased in response to exercise. For lumbar spine BMD, there was a net 1.4–1.5% increase in all treatment groups relative to controls (all $p<0.01$). There were no main effects of fortified milk at any skeletal site.
Conclusion A multi-component community-based exercise program was effective for increasing FN BMD in older men, but additional calcium–vitamin D_3 did not enhance the osteogenic response.

Keywords BMD · Calcium · Exercise · Fortified milk · Men · Vitamin D_3

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Introduction

Osteoporosis and related low trauma fractures are now recognised as a major public health concern in men. It is estimated that one in three men aged over 60 years will suffer an osteoporotic fracture in their lifetime [1]. Since there is no cure for this disease, there is an urgent need to identify safe, inexpensive and widely accessible evidence-based strategies to prevent and manage osteoporosis. Exercise and calcium–vitamin D are recognised as key modifiable lifestyle factors that have been shown to be important for the prevention and management of osteopo-

rosis, particularly in older women. A number of randomised controlled trials conducted in postmenopausal women have shown that exercise, particularly high intensity progressive resistance training (PRT) and/or weight-bearing impact exercise [2–5], and supplementation with calcium and/or vitamin D [6–10], can produce small gains (1–3%), or attenuate age-related losses in bone mineral density (BMD) at the hip and spine. While the mechanism of action of exercise and calcium–vitamin D on BMD are different—exercise has a site-specific modifying effect whereas calcium–vitamin D has a permissive systemic effect—there is some evidence that the skeletal benefits of exercise may be dependent on adequate dietary calcium intakes (>1,000 mg/day) [11]. However, in older adults there have been few studies specifically designed to examine whether increased dietary calcium, either alone or combined with vitamin D, can enhance the adaptive response to exercise. In the only factorial design trial to have been conducted in older adults, a significant exercise-by-calcium interaction was detected for femoral neck BMD in postmenopausal Chinese women with habitually low calcium intakes (<275 mg/day) [12]. Two other intervention trials, both conducted in older women, have also indicated a potential beneficial effect of combining increased dietary calcium with exercise on BMD [13, 14], but neither of these studies used a factorial study design. The aim of this 12-month, factorial design randomised controlled trial was to investigate whether milk fortified with calcium and vitamin D_3 combined with high intensity PRT and weight-bearing impact exercise could lead to a greater effect on BMD at loaded sites than the sum of each factor alone in community-dwelling men aged over 50 years.

Materials and methods

Participants

Healthy community-dwelling Caucasian men aged 50 to 79 years were recruited from within the local community in Geelong and surrounding areas, Victoria, Australia. Participants were excluded if they had taken calcium and/or vitamin D supplements or had participated in resistance training and/or high-impact weight bearing activities for greater than three times per week in the preceding 6 months, had a body mass index (BMI) of >35 kg/m^2, had a history of osteoporotic fracture or any medical condition or medication use known to affect bone metabolism, were lactose intolerant, consumed more than four standard alcoholic drinks per day, were current smokers, or had any chronic condition that might limit their ability to be involved in the intervention. A total of 451 men were pre-screened (via telephone), from which 296 were invited to have a DXA hip BMD scan. Men with normal to below average BMD (total hip or femoral neck T-score between +0.4 and −2.4 SD) were included in the study (n=180). All eligible men were required to obtain a medical clearance from their local physician to ensure that they were free of any contraindicated medical conditions to exercise based on the American College of Sports Medicine guidelines [15]. Reasons for exclusion are shown in Fig. 1. The study was approved by the Deakin University Human Ethics Committee and Barwon Health Research and Ethics Advisory Committee, and written consent was obtained from all participants.

Study design

This 2×2 factorial design study was a 12-month randomised controlled trial. The two factors were exercise and calcium–vitamin-D_3-fortified milk, each tested on two levels so that the 180 participants were randomly allocated to one of the four groups: (1) exercise + fortified milk (n=45); (2) exercise alone (n=46); (3) fortified milk alone (n=45); or (4) a control group (n=44). Prior to randomisation, participants were stratified according to age (<65 or

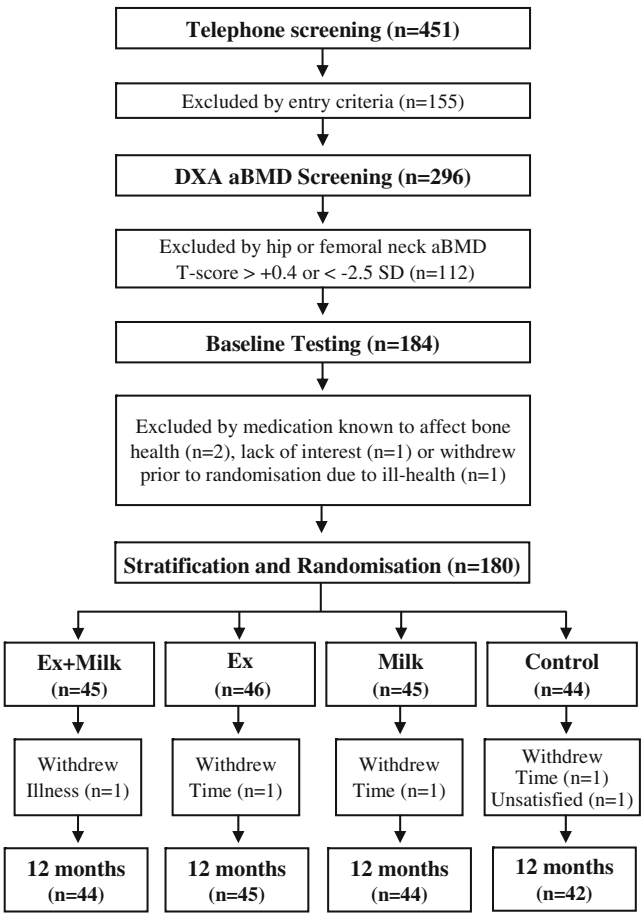

Fig. 1 Study profile

≥65 years) and baseline dietary calcium intake (<800 or ≥800 mg/day).

Exercise training

Participants randomised to the exercise program were asked to train on three non-consecutive days per week in one of four community leisure facilities under the supervision of qualified exercise trainers (maximum ratio of participants to trainer 8:1). Each exercise session lasted 60–75 min and consisted of warm-up and cool down activities, PRT, core muscle stabilisation exercises, and a series of moderate impact weight-bearing activities interspersed between the resistance training exercises. The 12-month periodised training program was divided into four distinct 12-week mesocycles, each with three, four weekly microcycles which were designed to be progressively more challenging. During the first 4 weeks of the program, all exercise sessions were supervised to ensure correct lifting and landing techniques and to monitor the appropriate amount of exercise and rest intervals. Thereafter, one session per week was supervised to provide ongoing personal attention, tuition and supervision. For the remaining two sessions, participants were instructed to seek assistance from local trained gymnasium staff when needed. Exercise compliance was computed from daily exercise cards completed by the men at the gymnasium and checked by records completed daily by the trainers that were returned to the research staff every month. The personal trainers also recorded any adverse events or injuries associated with the program.

The PRT was designed to be moderate to high in intensity and included a combination of machine and free weights and core muscle stabilisation exercises. The primary exercises used throughout the program focussed on major muscle groups with attachments on or near the hip and spine. This included squats (or leg press), lunges, hip abduction and adduction, latissimus dorsi pull down (or seated row), back extension and a combination of abdominal and core muscle stabilisation exercises. Additional exercises, including leg extension, calf raises, bench press, military press, bicep curls, tricep extension and lumbo-pelvic and spine stabilisation exercises, were also rotated throughout the program to ensure the development of muscle balance. During the first 12-week introductory mesocycle, participants completed three sets of 15–20 repetitions at 50–60% of their one repetition maximum (1-RM) strength. Thereafter, the training volume was set at two sets of eight to 12 repetitions. This included one warm-up set at 60–65% of 1-RM and one training set at 60–85% of 1-RM. For the first 4 weeks of each mesocycle, the training set was set at an intensity of 60–70% of 1-RM, which increased to 80–85% for the remaining 8 weeks of each mesocycle. All participants were instructed to perform each repetition in a slow, controlled manner, with a rest of 1–2 min between sets.

The weight-bearing impact component of the program was designed to load the lower extremities. For each 12-weekly mesocycle, three impact exercises were interspersed between the resistance training exercises. Participants were initially required to complete three sets of ten repetitions for each exercise, which progressively increased to a maximum of 20 repetitions at the completion of each mesocycle. Thus, the total number of impacts per session ranged from 90 to 180. The magnitude, rate and distribution (direction) of loads applied to the lower body were also progressively increased throughout the program by either increasing the height of jumps and/or by introducing more complex movement patterns. Prior to the commencement of the intervention, we conducted a pilot study to assess the peak vertical ground reaction forces (GRF) and time to peak force of 20 different weight-bearing exercises using the Leonardo mobile force plate (Novotech GmbH, Pforzheim, Germany). These exercises included single and double foot multi-directional landings, bench stepping and jumping off 15 and 30 cm boxes. The peak vertical GRFs varied from a minimum of 1.5 times body weight (BW) for walking on the spot with knee lifts, to a maximum of 9.7 times BW for a forward leap off a 30-cm bench with a rebound. The time to peak vertical forces ranged from 4 to 59 ms, with shorter times associated with the higher impact activities.

Calcium–vitamin-D_3-fortified milk

Participants assigned to the fortified milk protocol were asked to consume 400 mL per day (2×200 mL tetra packs) of reduced fat (~1%) ultra high temperature (UHT) milk, specifically formulated by Murray Goulburn Co-operative Co. Ltd (Brunswick, Australia). As previously reported [16], each 200 mL milk tetra pack contained approximately 500 mg of calcium and 400 IU of vitamin D_3, 418 kJ energy, 6.6 g protein, 2.2 g fat, 11 g lactose, 100 mg sodium and 250 mg phosphorous. The milk was fortified with a calcium salt derived from fresh milk whey. The vitamin D (vitamin D_3) that was used to fortify the milk was obtained from DSM Nutritional Products Pty Ltd (NSW, Australia). Four batches of the milk were produced over the 12-month intervention with participants receiving a new batch every 3 months. The calcium and vitamin D_3 levels of each batch were analysed by Murray Goulburn before being distributed. The average (±SD) calcium and vitamin D_3 levels per 100 mL for the four batches were 251 ±21 mg and 191±34 IU, respectively. Participants recorded the number of tetra packs consumed per day on compliance calendars, which were collected and checked every 3 months. Compliance was calculated as a percentage by dividing the number of tetra packs consumed by the expected consumption each month and multiplied by 100.

Anthropometry, bone mineral density and body composition

Height was assessed using a Holtain wall stadiometer (Crymych, Dyfed). Weight was measured using an A&D UC-321 electronic scale to the nearest 0.1 kg. BMI was calculated as body weight (kilogram) divided by height (metre) squared (kilogram per square metre). Lumbar spine (L_1–L_4), total hip, femoral neck (total, upper and lower region) and trochanter areal BMD (aBMD) and total body lean mass and fat mass were assessed by DXA (Prodigy, GE Lunar Corp., Madison, WI, USA), with analysis software version 8.10.027. The upper neck region of interest (ROI) includes everything above the hip axis length line whereas the lower neck ROI represents everything below this line. The short term co-efficient of variation (CV) for repeated measurements of lumbar spine and proximal femur aBMD in our laboratory ranged from 0.6% to 1.0%. The CVs for total body lean mass and fat mass were 0.7% and 1.0%, respectively.

Muscle strength

Before the determination of upper and lower body one repetition maximum (1-RM) muscle strength, participants attended two separate familiarisation sessions where they were shown correct exercise techniques by a trained instructor and given the opportunity to become accustomed to the selected exercises. To determine 1-RM, each participant initially performed a warm-up set of eight repetitions with a light load. Following the successful completion of a further five to six repetitions at a heavier weight selected by the instructor and after a brief rest (~2 min), the workload was increased incrementally until only one repetition with correct technique could be completed. The following exercises (leg press, latissimus dorsi pull down and bench press) were used to document changes in upper and lower body muscle strength.

Hormonal and biochemical measurements

Fasting, morning (8–10 AM) blood samples (10 mL) were obtained from each participant's antecubital vein at baseline and 12 months. All serum samples were subaliquoted and stored at −80°C until assayed. All assays were performed in duplicate, and each participant's samples were assessed in a single batch. Serum intact PTH (hPTH 1-84) was measured by an immunoradiometric assay (IRMA) using the DiaSorin N-tact PTH IRMA kit (DiaSorin Inc, Stillwater, MN, USA). The mean intra- and interassay coefficients of variation were 9.4% and 18.9%, respectively. Serum 25-hydroxyvitamin D [25-OHD_3] was measured using a DiaSorin immunoassay (RIA; Stillwater, MN, USA). The mean interassay CV ranged from 3.9% to 5.8%. Serum total calcium, phosphate, albumin, creatinine and alkaline phosphatase (ALP) were simultaneously assessed at both time points using a RX Daytona automated chemistry analyser (Furuno Electronic Co. Ltd, Japan). The interassay CVs ranged from 3.4% to 10.4%.

Diet, physical activity and medication use

Nutrient intakes were assessed using a 3-day food diary (two weekdays and one weekend day), with the option of weighing items and analysed using the Foodworks nutrient analysis software program (Xyris Software, Brisbane, Queensland, Australia). All participants were provided with detailed verbal and written instructions for completing their food diaries. Leisure time and habitual physical activity outside of the exercise intervention (hours of weight-bearing exercise per week) were assessed using the CHAMPS Physical Activity Questionnaire [17]. Information on medication use (including calcium–vitamin D supplementation) was determined by questionnaire and confirmed by interview.

Statistical analysis

Statistical analyses were conducted using Stata Statistical Software release 8.0 (Stata, College Station, TX, USA). Baseline characteristics between the groups were compared using analysis of variance. Pooled time series regression analysis for longitudinal data was used to test for an interaction between exercise and calcium–vitamin D_3. If no significant interactions were detected, the main effects of exercise (exercise + fortified milk and exercise alone vs fortified milk and control) and calcium–vitamin-D_3-fortified milk (exercise + fortified milk and fortified milk vs exercise and controls) were examined. Data were initially analysed for all participants who enrolled in the study and completed the 12-month follow-up testing (intention-to-treat analysis). However, in order to determine whether more regular exercise participation influenced the changes in aBMD, per protocol analyses were subsequently conducted excluding men who did not attend the training sessions regularly. A percentage exercise attendance of ≥60% was chosen as the cutpoint to define 'regular attendance' as there was a natural separation in the class attendance at this value; 71% of the men had an attendance level above this mark. In this analysis, the number of men in the exercise group was reduced by 26 (i.e. n=65). Serum 25-OHD_3, albumin and calcium were log-transformed prior to analyses. Between-group differences were calculated by subtracting within-group changes from the baseline values in each group for each parameter. Separate models were used to assess the within-group changes, which were

expressed either as absolute changes or as a percentage changes from baseline. Percentage changes in the log-transformed 25-OHD_3 represents the absolute differences from baseline multiplied by 100. All data are presented as means ± SD or 95% CI unless stated.

Results

Baseline characteristics

There were no significant between-group differences for any of the baseline characteristics (Table 1), with the exception that back and leg muscle strength was greater in the control relative to exercise or exercise + fortified milk groups. The average dietary calcium intake for all participants was 1,002±397 mg/day, but 58% of the men had a calcium intake below the current Australian recommended dietary intake (RDI) of 1,000 mg per day for men aged 51 to 70 years [18]. At study entry, the mean serum 25-OHD_3 level was 86.2±35.9 nmol/L; no participants had severe vitamin D deficiency [25-OHD_3 <12.5 nmol/L]; one participant had moderate deficiency [25-OHD_3 12.5–25 nmol/L] and 17 participants (9.4%) had mild deficiency [25-OHD_3 25–50 nmol/L].

Study attrition and compliance

Five of the 180 men (2.8%) withdrew from the study over the 12-month period [exercise + fortified milk n=1; exercise n=1; fortified milk n=1; control n=2]. The reason for withdrawal included: illness unrelated to the study (n=1); work or personal time commitments (n=3), and dissatisfaction with group allocation following randomisation (n=1). The average compliance with the exercise program was 67% (95% CI 61, 73%), and did not differ between the exercise + fortified milk and exercise alone group (69% and 65%). The average compliance with fortified milk was 90% (95% CI 86, 93%), and was no different between the exercise + fortified milk and fortified milk group (92% and 87%).

Adverse events

There were no serious injuries or adverse events associated with the exercise program. The limited number of minor injuries included exacerbation of longstanding gout of the

Table 1 Baseline characteristics of the participants according to treatment group (mean ± SD)

Characteristics	Exercise + milk (n=45)	Exercise (n=46)	Milk (n=45)	Control (n=44)
Age (years)	61.7±7.6	60.7±7.1	61.7±7.7	59.9±7.4
Height (cm)	174.3±6.3	174.2±6.6	174.4±5.8	175.0±6.6
Body composition				
Weight (kg)	83.2±11.9	85.2±10.9	84.1±9.8	81.9±10.7
Lean mass (kg)	57.0±6.4	58.5±6.5	56.6±5.3	57.5±5.8
Fat mass (kg)	22.9±8.7	23.5±6.6	24.2±7.4	21.2±7.5
Physical activity				
Weight-bearing activity (h/week)	3.7±3.9	3.6±3.4	3.3±3.8	3.4±4.1
Muscle strength				
Chest, bench press (kg)	49.1±12.7	55.0±13.1	49.1±13.5	52.7±11.8
Back, lat. pull down (kg)	61.4±14.5	65.8±11.7	66.3±11.2	68.7±12.1*
Legs, leg press (kg)	63.4±18.0	64.7±16.5	71.4±13.7	74.4±18.1**
BMD (g/cm^2)				
Femoral neck (FN)	0.922±0.072	0.938±0.080	0.919±0.076	0.933±0.084
Upper FN	0.749±0.786	0.767±0.952	0.747±0.084	0.756±0.097
Lower FN	1.090±0.089	1.099±0.090	1.088±0.094	1.106±0.098
L_1–L_4	1.231±0.163	1.247±0.140	1.206±0.146	1.238±0.169
Total hip	1.026±0.079	1.022±0.092	1.004±0.085	1.010±0.115
Trochanter	0.907±0.084	0.892±0.093	0.879±0.091	0.886±0.117
Biochemistry and hormonal				
Calcium (mmol/L)	2.39±0.18	2.40±0.16	2.39±0.16	2.41±0.18
Phosphorus (mmol/L)	1.03±0.17	1.03±0.14	1.02±0.13	1.04±0.17
Albumin (g/L)	43.3±3.4	44.0±2.9	44.0±3.9	44.3±4.0
Creatinine (mmol/L)	107.9±20.0	103.4±13.6	105.9±14.6	103.3±15.0
ALP (U/L)	63.7±18.9	67.9±19.4	68.3±18.7	65.9±20.1
PTH (pg/mL)	26.1±12.2	27.7±13.3	27.1±10.2	25.3±9.8
25-OHD_3 (nmol/L)	90.5±29.9	85.0±40.6	83.6±32.7	85.7±40.3

*p<0.05 vs exercise alone; **p<0.05 vs exercise + milk and exercise alone

foot (n=1), aggravated knee or hip pain (n=2; both were able to continue with exercise after program modification), lower back injury (n=2; one recovered with 2-weeks rest; one withdrew due to aggravated pain associated with a longstanding prolapsed disc) and aggravation of long-standing shoulder injury (n=2; both participants returned to exercise following treatment). Three men were diagnosed with an inguinal hernia, but all were able to continue with the exercise program following treatment.

Changes from baseline over 12 months

In both the ITT and per protocol analysis, there was no evidence to suggest that consumption of fortified milk enhanced the effect of exercise on any of the outcome variables (Table 3). Therefore, the between-group differences (main effects) for exercise and fortified milk and within-group changes are described separately.

Diet and physical activity

Total energy and fat intake and the percentage of energy derived from fat were no different between the exercise and fortified milk groups (Table 2). As expected, dietary intakes of calcium, vitamin D and protein (grams per day and the percentage of energy from protein) increased significantly in the fortified milk groups, and were greater than in the non-supplemented groups (p ranging from <0.01 to <0.001). On average, dietary calcium, vitamin D and protein intake were 691 mg/day, 18 μg/day and 14 g/day greater in the fortified milk groups (all p<0.001) after 12 months. Leisure and recreational weight-bearing physical activity did not change within or between the exercise and fortified milk groups throughout the intervention.

Body composition

Weight and fat mass increased by 1.0 kg (p<0.01) and 0.6 kg (p=0.07) in the fortified milk group compared to the non-supplemented groups, respectively (Table 3). This was largely attributed to a significant increase in the fortified milk alone group; neither weight nor fat mass increased significantly in the exercise + fortified milk group. In the exercise relative to non-exercise groups, total body lean mass increased by 0.6 kg (p<0.05) and fat mass decreased by 0.8 kg (p<0.05).

Bone mineral density

Exercise resulted in a significant 1.8% net gain in femoral neck aBMD relative to no-exercise after 12 months (p<0.001; Fig. 2 and Table 3). Similar results were observed for both upper and lower femoral neck aBMD (2.0% and 1.7%,

Table 2 Mean (±SD) baseline values and the absolute unadjusted changes (95% CI) from baseline for the dietary characteristics within each group

Characteristics	Exercise + milk (n=45)		Exercise (n=46)		Milk (n=45)		Control (n=44)	
	Baseline	Change	Baseline	Change	Baseline	Change	Baseline	Change
Energy (kJ/day)	9,694±2,149	461 (−390, 1,313)	9,884±1,948	250 (−525, 1,026)	9,761±1,717	846 (84, 1,609)*	10,199±2,201	−318 (−1,079, 442)
Fat (g/day)	84±29	4 (−8, 15)	83±23	−3 (−15, 8)	86±21	9 (−2, 19)	87±26	−3 (−13, 7)
Percent energy fat	32±7	0.4 (−2.2, 3.0)	31±5	−1.7 (−4.3, 0.9)	33±5	0.2 (−2.1, 2.5)	32±5	−0.1 (−2.1, 1.9)
Protein (g/day)	103±25	17 (6, 28)**	110±24	6 (−4, 15)	102±19	15 (8, 22)**	107±27	−4 (−13, 6)
Percent energy protein	18±3	1.8 (0.4, 3.1)**	19±3	0.5 (−1.2, 2.1)	18±3	1.1 (0.0, 2.1)*	18±3	0.03 (−1.4, 1.4)
Calcium (mg/day)	911±360	827 (677, 977)**	1,064±449	21 (−83, 127)	1,039±455	682 (537, 828)**	996±293	46 (−90, 181)
Vitamin D (μg/day)	1.2±2.1	19.1 (16.7, 21.4)**	0.8±1.1	1.1 (0.6, 2.2)*	1.4±3.0	18.3 (16.2, 20.3)**	0.7±1.0	1.1 (0.0, 2.2)*

*p≤0.05; **p<0.001 within-group change from baseline

Table 3 Mean absolute or percentage unadjusted changes (95% CI) from baseline for body composition, BMD, serum parathyroid hormone (PTH) and 25-OHD_3 within each group and the mean differences (95% CI) between the exercise and calcium–vitamin-D_3-fortified milk groups (main effects)

Characteristics	Exercise + milk (n=45)	Exercise (n=46)	Milk (n=45)	Control (n=44)	Interaction (p value)	Main effects	
						Exercise	Milk
Body composition[a]							
Weight	0.6 (−0.1, 1.4)	0.0 (−0.8, 0.8)	1.3 (0.7, 2.0)#	0.0 (−0.6, 0.6)	0.45	−0.4 (−1.1, 0.3)	1.0 (0.3, 1.7)***
Lean mass	1.0 (0.4, 1.5)#	0.7 (0.2, 1.1)***	0.4 (0.0, 0.9)	0.1 (−0.4, 0.5)	0.98	0.6 (0.1, 1.0)**	0.3 (−0.1, 0.8)
Fat mass	−0.3 (−1.0, 0.3)	−0.7 (−1.6, 0.1)*	0.7 (0.2, 1.3)**	−0.1 (−0.6, 0.4)	0.78	−0.8 (−1.5, −0.2)**	0.6 (0.03, 1.3)*
BMD[b]							
FN	1.4 (0.5, 2.3)#	1.7 (0.8, 2.6)#	−0.4 (−1.1, 0.3)	−0.2 (−0.9, 0.6)	0.96	1.8 (1.0, 2.6)#	−0.3 (−1.1, 0.6)
Upper FN	1.6 (0.3, 2.9)**	1.8 (0.5, 3.1)***	−0.2 (−1.7, 1.3)	−0.5 (−1.8, 0.8)	0.87	2.0 (0.8, 3.3)***	0.1 (−1.3, 1.4)
Lower FN	1.3 (0.4, 2.3)***	1.8 (0.7, 2.9)#	−0.4 (−1.1, 0.4)	0.0 (−0.8, 0.8)	0.87	1.7 (0.8, 2.6)#	−0.4 (−1.3, 0.5)
L_1–L_4	2.0 (1.2, 2.8)#	2.1 (1.2, 3.0)#	2.1 (1.1, 3.0)#	0.6 (−0.1, 1.3)	<0.05	Exercise + milk, exercise, milk > controls (all p<0.01)	
Total hip	1.1 (0.4, 1.7)#	1.2 (0.7, 1.8)#	1.2 (0.7, 1.8)#	0.5 (0.0, 1.0)	0.19	0.3 (−0.3, 0.8)	0.3 (−0.3, 0.9)
Trochanter	1.1 (0.4, 1.9)***	1.6 (0.7, 2.4)#	1.6 (0.7, 2.5)#	0.8 (0.0, 1.5)	0.16	0.2 (−0.6, 1.0)	0.2 (−0.6, 1.0)
Hormonal[b]							
25-OHD_3	7.4 (−3.7, 18.4)	−16.0 (−27.9, −4.1)**	15.3 (5.8, 24.8)***	−7.2 (−13.9, −0.5)	0.93	−8.5 (−18.9, 1.9)	23.0 (13.1, 32.8)#
PTH	−1.7 (−8.7, 5.4)	−1.8 (−11.7, 8.1)	2.2 (−8.5, 12.8)	5.8 (−4.2, 15.7)	0.90	−5.7 (−14.9, 3.6)	−1.7 (−11.0, 7.6)

*p=0.07; **p≤0.05, ***p<0.01, #p<0.001 within-group change from baseline or between-group differences
[a] Absolute changes (in kilograms) from baseline
[b] Percentage changes from baseline. Changes in serum 25-OHD_3 represent log-transformed data

respectively). For total hip aBMD, there was a significant 1.1% to 1.2% within-group increase in both exercise groups, but there was no main effect of exercise. This is likely due to the significant 1.2% within-group increase in hip aBMD in the fortified milk alone group. Comparable results were observed for both trochanter and lumbar spine (L_1–L_4) aBMD, but for the latter there was a significant net 1.4% to 1.5% increase in aBMD in all three treatment groups compared to the control group. In the subsequent per protocol analysis, the exercise main effects remained significant at all femoral neck sites [femoral neck 1.9%; upper femoral neck 2.2%; lower femoral neck 1.7% (p ranging from <0.01 to <0.001], but between-group differences in favour of the exercise group were also detected at both the total hip (net gain 0.5%, p<0.05) and trochanter (net gain 0.6%, p=0.06). There were no main effects of fortified milk at any skeletal site, even after adjusting for changes in weight.

Muscle strength

Exercise led to 20% to 52% improvements in both upper and lower body muscle strength relative to no exercise after 12 months (p<0.001; Fig. 3). Within-group analysis revealed that all muscle strength measures at each time point increased significantly relative to baseline in the exercise groups (p ranging from <0.01 to <0.001), but the greatest increases occurred after 6 months training for the back muscles and 12 months training for both the chest and legs. There were no main effects of fortified milk on any muscle strength measure.

Hormonal and biochemical parameters

Serum 25-OHD_3 levels increased by 23% in the fortified milk group compared to non-supplemented groups (p< 0.001; Table 3). This was due to a mean 11.4% increase in the fortified milk group and a 11.6% reduction in the non-supplemented groups. No significant main effect of fortified milk on serum parathyroid hormone (PTH) was detected. Similarly, there was no main effect of exercise for either serum 25-OHD_3 or PTH. For all other biochemical parameters, there was no significant main effect of either exercise or fortified milk, except for serum ALP for which there was a main effect of the fortified milk (−5.6%; p<0.05) and exercise (5.6%; p=0.07).

Discussion

The main finding from this 12-month, factorial design RCT was that supplementation with 1,000 mg of calcium and 800 IU of vitamin D_3 per day did not enhance the effects of exercise on aBMD at any skeletal site in healthy, community-dwelling men aged over 50 years. However, exercise (with or without calcium–vitamin D_3) led to a ~1.8% greater gain in femoral neck aBMD and a significant increase in lean mass and muscle strength compared to no-

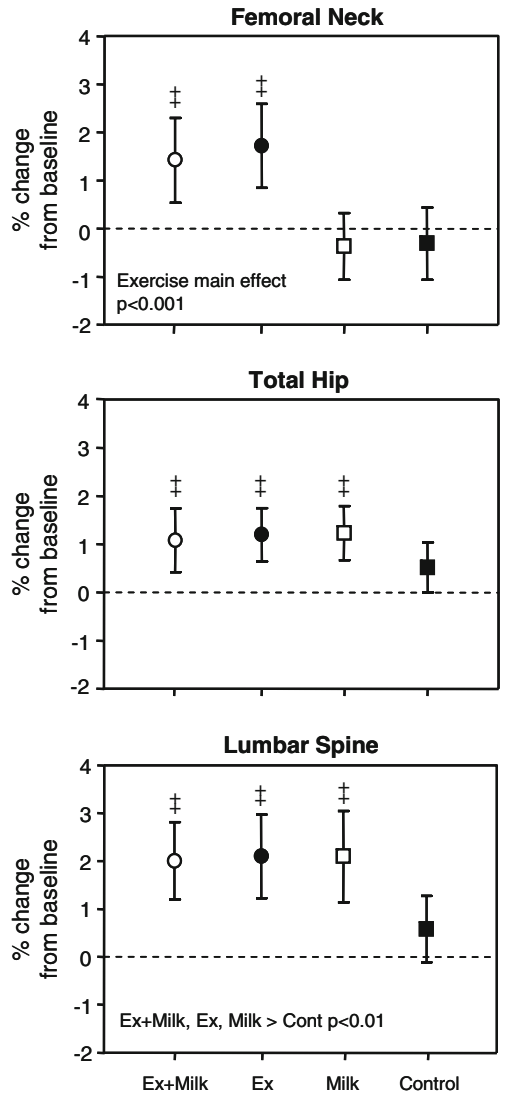

Fig. 2 Mean unadjusted percentage change (95% CI) in femoral neck, total hip and lumbar spine (L_1–L_4) aBMD in the Ex + Milk (n=45), Ex (n=46), Milk (n=45) and control groups (n=44). At the femoral neck, there was a significant main effect of exercise (p<0.001). The increase in L_1–L_4 aBMD in the treatment groups was significantly greater than the increases in the control group (all p<0.01). ‡p<0.001 within-group change from baseline

exercise. At the lumbar spine, exercise and fortified milk alone or in combination resulted in a similar ~1.5% net gain in BMD relative to controls.

We also found that increased compliance to training (≥60% attendance at prescribed sessions) was associated with significant improvements in total hip and trochanter BMD compared to no-exercise. However, there was no main effect of the fortified milk on aBMD at any site.

In pre- and postmenopausal women, a number of studies have shown that moderate to high intensity progressive resistance training and weight-bearing impact exercise alone or in combination can lead to small increases in aBMD or prevent age-related bone loss at loaded sites [5, 19–22]. However, few intervention studies have examined the effects of different modes of exercise on aBMD in older men. In this study, we have shown that a community-based, multi-component exercise program involving high intensity PRT in combination with moderate-to-high impact weight-bearing exercise was effective for increasing femoral neck aBMD in men aged over 50 years. Furthermore, increased compliance to the training, equivalent to approximately two sessions per week, was associated with significantly greater gains in trochanter and total hip aBMD relative to the non-exercise group. These changes in proximal femur aBMD were similar to those observed in postmenopausal women

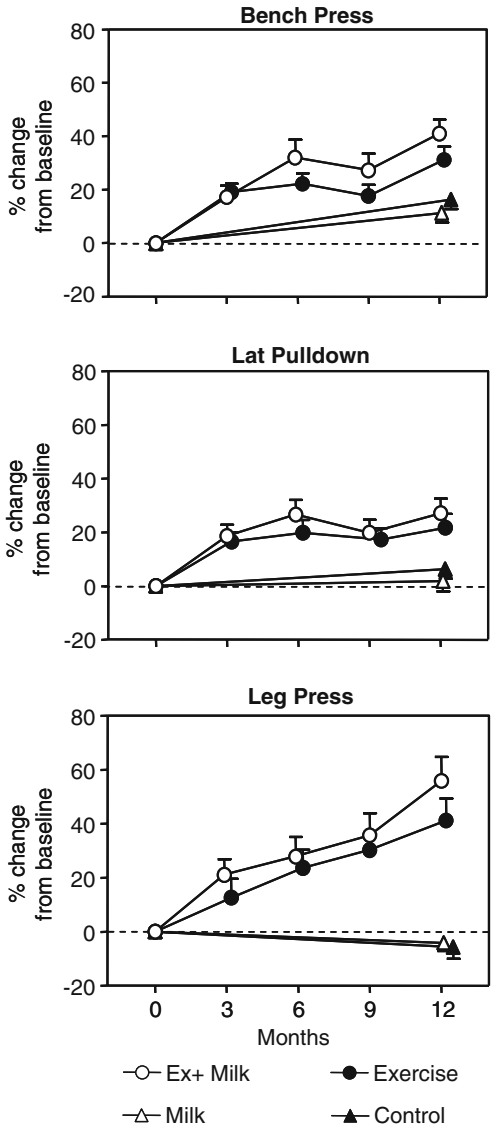

Fig. 3 Mean percentage changes (±SE) from baseline for upper body (bench press), back (lat. pull down) and lower body (leg press) muscle strength according to treatment group. Exercise resulted in significant increases in all strength measures relative to both baseline and the non-exercise group (main effects, p<0.001)

following a similar multi-component exercise program. Engelke et al. [21] reported that a 36-month intervention incorporating high intensity PRT (70–90% of 1-RM) and lower extremity impact exercise (running games, skipping, multi-directional jumping) performed four times per week led to a ~2% net benefit in total hip aBMD. Together, these findings indicate that a targeted multi-component lower extremity exercise program can improve hip bone density in older adults.

There are several factors which may account for the beneficial effect on proximal femur aBMD in our study. Firstly, 97% of the men completed the study and exercise compliance averaged 67% throughout the 12-month intervention. Secondly, the training program was designed to incorporate the key loading characteristics known to positively affect bone [23–25]. That is, it was site specific and included rapidly applied loads that were moderate to high in magnitude and were novel or unusual in their distribution. In particular, the PRT program specifically loaded the psoas major, iliacus, piriformis and gluteus medius and minimus muscles, all of which attach to the greater or lesser trochanter of the hip. The weight-bearing impact exercises were also designed to generate moderate to high magnitude loads (strains), which were applied rapidly and from unusual directions. Quantification of the average peak vertical GRFs associated with these exercises revealed that they progressed from 1.5 BW for walking on the spot to 9.7 BW for jumping off a 30-cm step. Several previous studies in premenopausal women have reported that multi-directional weight-bearing exercises (e.g. aerobic jumping, bench stepping, drop jumps) which produce peak forces ranging from 2.1 to 5.6 BW or accelerations exceeding 3.5 to 3.9 g were effective for improving proximal femur aBMD [26–28]. Finally, the exercise program in our study was designed to be progressive which is important because the adaptive skeletal response to loading only occurs when a given load (strain) exceeds a certain threshold [minimum effective strain (MES)] or 'set-point' [29]. Once bone adapts to this given level of strain, progressively greater loads are required to stimulate further bone adaptation. We designed a periodised exercise program that was divided into four distinct 12-weekly mesocyles, each with three 4-weekly microcycles that were progressively more challenging in order to gradually overload the musculoskeletal system. Importantly, there were no major injuries or adverse events associated with the program, which was well tolerated by the men and also resulted in significant increases in muscle strength and muscle mass.

A unique component of our study was that we assessed aBMD within the superior and inferior regions of the femoral neck. Given that loading stresses generated during normal gait are concentrated primarily within the inferomedial region of the femoral neck, we hypothesised that the exercise program would lead to greater adaptation at the inferior region [30, 31]. However, we found that there was a similar significant exercise induced increase for both upper and lower femoral neck aBMD (2.0% and 1.7%, respectively). While it is difficult to explain this finding, it may be that the MES loading threshold is different for the upper and lower femoral neck region. The cortices are thinner at the superior portion of the femoral neck [30, 32], so it is possible that this region has a lower threshold level for adaptation, which may be accentuated by increased age-related bone loss on the endosteal surface [33]. This notion of a variable MES threshold is supported by the results of a study by Hsieh et al. [34], which showed that the strain threshold for osteogenesis was variable at different bone sites and was greater distally compared to proximally in the ulna of female rats. While it is likely that our loading program generated lower magnitude stresses and strains at the upper femoral neck, it is possible that there is a lower threshold at this site which may have contributed to the similar aBMD changes relative to the lower neck region.

At the lumbar spine, both exercise and calcium–vitamin-D-fortified milk resulted in a net benefit of around 1.5% in aBMD relative to controls. The positive effects of exercise at the spine are consistent with a number of intervention studies and meta-analyses in older women which have reported a significant beneficial effect of high intensity PRT on lumbar spine aBMD [5, 19, 21, 35]. The major mechanism by which resistance training is likely to contribute an osteogenic response at this site is via the action of muscle pull and the forces generated during muscle contraction at the tendon attachment on the bone [3]. Our exercise program was specifically designed to load the muscles that originate from or insert on the lumbar vertebra (psoas major, quadratus lumborum and the back extensor muscles), all of which load the spine. The finding that the fortified milk also resulted in net 1.5% gain in aBMD at this site is consistent with numerous other studies in older men and women which have also reported beneficial effects following calcium and/or vitamin D supplementation after 12 months [7, 16, 36].

The finding that consumption of milk fortified with 1,000 mg of calcium and 800 IU of vitamin D_3 per day for 12 months did not enhance the effect of exercise on BMD at any site is likely to be attributed to the high baseline calcium intakes and sufficient serum 25-OHD_3 levels of the men in our study. While the level of dietary calcium or serum 25-OHD_3 necessary to optimise BMD in the presence of exercise is not known, the mean baseline calcium intakes and 25-OHD_3 levels of the men in our study ranged from 911 to 1,039 mg/day and 83.6 to 90.5 nmol/L across the four groups, respectively. These levels are similar to the current recommended calcium intake of 1,000 mg/day for Australian men aged 51 to

70 years and higher than the current recommendations for adequate circulating 25-OHD_3 of ~75 nmol/L [37].

There is no reason to suggest that calcium in excess of requirements or circulating 25-OHD_3 levels higher than optimal should build a stronger bone in response to a given amount of loading [38]. Supplemental calcium–vitamin D tends to slow age-related bone loss by downregulating bone remodelling rather than by sustaining a positive remodelling balance [39]. It is more likely that exercise-induced increases in BMD require sufficient calcium–vitamin D, and that bone adaptation may be compromised by inadequate intakes. In support of this notion, the findings from a similar intervention in Chinese postmenopausal women with habitually low calcium intakes (<275 mg/day) found that supplementation with 800 mg/day of calcium for 10 months did enhance the effects of exercise on femoral neck aBMD [12]. The results from animal studies have also shown that bone's adaptive response to loading is compromised by calcium insufficiency [39]. In postmenopausal women, Prince et al. [14] reported that increasing dietary calcium intakes from around 800 to 1,800 mg/day for 2 years in combination with exercise reduced the rate of bone loss at the femoral neck compared with calcium supplementation alone. In this study, the supplemental calcium corrected insufficient baseline intakes; the average calcium intake was 60% of the RDI (1,300 mg/day) for Australian postmenopausal women. In our study, men receiving the fortified milk increased their mean dietary calcium intakes by a similar extent (mean change 950 to 1,750 mg/day), but the higher baseline intakes were equivalent to the current RDI for older Australian men (1,000 mg/day), and thus arguably sufficient to meet current requirements.

The strength of our study lies in its randomised controlled design, relatively long-term follow-up and high study retention and compliance to the intervention. The novel aspect is that it is the first trial to examine the combined and independent effects of a multi-component exercise program and supplementation with calcium–vitamin-D_3-fortified milk on BMD in older men. However, there are several limitations. First, our sample size was relatively small to detect a significant exercise-by-calcium–vitamin D_3 interaction. Initial power calculations based on the limited data available in women indicated that we required 58 men per group [14]. Despite an extensive recruitment campaign, we were only able to recruit 180 men, which can be partly attributed to our relatively stringent inclusion/exclusion criteria. Second, we recruited relatively healthy, ambulatory community-dwelling men without osteoporosis or low dietary calcium or serum 25-OHD_3 levels. Further factorial design studies are required to uncover whether calcium–vitamin D_3 supplementation can enhance the effects of exercise on BMD in an 'at-risk' groups of older men.

In summary, we found that a community-based, multi-component exercise program incorporating high intensity PRT and weight-bearing impact exercise was effective for improving proximal femur aBMD, muscle strength and lean mass in older men. Importantly, the low attrition and exercise compliance coupled with the low number of adverse events indicates that this type of exercise program is safe, feasible and well tolerated by previously untrained, but otherwise healthy older men. Furthermore, since the exercise intervention was conducted in a community-based setting, the results from this study are likely to be generalisable to the broader community. However, we found no evidence to suggest that calcium–vitamin-D_3-fortified milk enhances the effects of exercise on aBMD, which is likely due to the adequate dietary calcium intakes and sufficient circulating 25-OHD_3 levels in our men. In conclusion, this study provides the evidence-base to support the development and implementation of future community based lifestyle programs incorporating multi-component exercise and healthy nutritional strategies to enhance musculoskeletal health and function in older men.

Acknowledgements This study was financially supported by a grant from the Australian Research Council (ARC) Linkage Scheme. Associate Professor Robin Daly was supported by an Osteoporosis Australia Research Fellowship and a National Health and Medical Research Council (NHMRC) Career Development Award (ID 425849). We thank Murray Goulburn Co-operative Co Ltd, in particular Peter Hobman and Michelle Rowney, for providing the calcium–vitamin D_3 low fat UHT-fortified milk used in the study. The authors thank The City of Greater Geelong and Ocean View Health Club for their generous provision of the gymnasium facilities used throughout the study. The authors also thank the following people for contributions to this study: Nicole Petrass, Joanne Daly, and Sam Korn.

Conflicts of interest None.

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