DISSERTATIONS IN
HEALTH
SCIENCES

MATTI KÄRKKÄINEN

Physical Capacity and Supplementation of Vitamin D and Calcium in Postmenopausal Women

PUBLICATIONS OF THE UNIVERSITY OF EASTERN FINLAND
Dissertations in Health Sciences

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To be presented by permission of the Faculty of Health Sciences, University of Eastern Finland for public examination in the Auditorium L22, Snellmania, University of Eastern Finland, on Saturday 29th January, at 12 noon

Publications of the University of Eastern Finland
Dissertations in Health Sciences
39

Bone and Cartilage Research Center (BCRU), University of Eastern Finland (Kuopio)
Department of Orthopaedics, Traumatology and Hand Surgery, Kuopio University Hospital
Kuopio 2011

Kopijyvä Oy
Kuopio, 2011

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http://www.uef.fi/kirjasto

ISBN: 978-952-61-0309-9 (print)
ISSN: 1798-5706 (print)
ISSNL: 1798-5706
ISBN: 978-952-61-0310-5 (pdf)
ISSN: 1798-5714 (pdf)

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Kärkkäinen, Matti. Physical capacity and supplementation of vitamin D and calcium in postmenopausal women. Publications of the University of Eastern Finland. Dissertations in Health Sciences 39. 2011. 100p.
ISBN: 978-952-61-0309-9 (print)
ISSN: 1798-5706 (print)
ISSNL: 1798-5706
ISBN: 978-952-61-0310-5 (pdf)
ISSN: 1798-5714 (pdf)

ABSTRACT

In general, the fracture risk is highest among those who have osteoporotic bone mineral density (BMD) but nonetheless most fragility fractures occur in subjects who do not have osteoporosis. Their poor physical condition might predispose these individuals both to low BMD and a higher risk for fall-related fractures.

Vitamin D has been shown to have also physiological importance outside of bone health and calcium homeostasis, and there is evidence that it plays a role in the prevention of falling and it has been associated with a wide spectrum of diseases.

The present thesis was based on Kuopio Osteoporosis Risk Factor and Prevention study (OSTPRE) and its substudy OSTPRE Fracture Prevention Study (OSTPRE-FPS). Physical capacity tests and fracture validation were performed on the OSTPRE cohort (n=2928). The OSTPRE-FPS was an open-label RCT with a 3-year duration conducted during 2003-6. The OSTPRE-FPS population of 3,432 women was randomly selected from the population-based OSTPRE cohort. The women were randomized into two groups of equal size. The intervention group (n=1,718) obtained daily cholecalciferol 800 IU and calcium carbonate 1000 mg supplementation divided into two daily doses for three years and the control group (n=1,714) received no supplementation. The physical capacity tests and BMD measurements were performed in a pre-defined subsample of 750 women. In addition, falls were self-reported from the entire trial population (n=3432).

It was shown that functional capacity was decreased in women with femoral neck osteoporosis (WHO classification) compared to women with normal or osteopenic BMD: standing-on-one-foot (SOOF) −39% ($p=0.001$), grip strength (GS) −18% ($p<0.001$), leg extension strength −19% ($p=0.007$) and ability to squat down on the floor −40% ($p=0.004$). Furthermore, the decreased GS, low leg extension strength, inability to perform SOOF 10 seconds and self-assessed ability to walk less than 100 meters were associated with future fractures. Accordingly, it was proposed that GS could be used in medical decision making to identify those women who would benefit from BMD measurements albeit alone it may not represent an accurate enough tool for osteoporosis screening. In addition, being unable to perform SOOF for 10 seconds, GS and a question about ability to walk less than 100 meters may help to predict postmenopausal fractures. It is speculated that the poor physical condition increased both the prevalence of low BMD and the risk for fall-related fractures.

The OSTPRE-FPS indicated that daily vitamin D 800IU and calcium 1000mg could decrease the risk of multiple falls requiring medical attention in a general population of women aged 65 to 71. In addition, vitamin D and calcium supplementation have a positive effect on the skeleton in women who have adequate nutritional calcium intake. These benefits were gained without any severe side effects from the supplementation. Accordingly, a higher vitamin D intake can be recommended for postmenopausal women aged 65 to 71.

National Library of Medicine Classification: QU 173, QV 276, WE 180, WE 200, WA 288
Medical Subject Headings: Bone Mineral Density, Calcium, Falls, Fractures, OSTPRE, Randomized Controlled Trial, Vitamin D

Kärkkäinen, Matti. Fyysinen toimintakyky ja D-vitamiini- ja kalsiumlisä vaihdevuosi-ikäisillä naisilla. Itä-Suomen yliopiston julkaisu. Terveystieteiden tiedekunnan väitöskirjat 39. 2011. 100s.
ISBN: 978-952-61-0309-9 (print)
ISSN: 1798-5706 (print)
ISSNL: 1798-5706
ISBN: 978-952-61-0310-5 (pdf)
ISSN: 1798-5714 (pdf)

TIIVISTELMÄ

Yleisesti ottaen murtumariski on suurin niillä henkilöillä joilla on osteoporoottinen luuntiheys. Kuitenkin suurin osa murtumista tapahtuu henkilöille, joilla ei ole osteoporoosia. Huono fyysinen kunto voi sekä altistaa alhaiselle luun mineraalitiheydelle että lisätä kaatumaperäisten murtumien riskiä.

D-vitamiinia tarvitaan luuston terveyden ylläpitämiseen ja elimistön kalsiumtasapainon säätelyyn, mutta sillä on muutakin fysiologista merkitystä. On viitteitä siitä, että D-vitamiinin riittävällä saannilla on merkitystä kaatumisten ehkäisyssä. Sen puute on yhdistetty myös moniin sairauksiin.

Tämä väitöskirja perustuu Kuopion Osteoporoosin vaaratekijät ja ehkäisy – tutkimukseen (OSTPRE) ja sen alaiseen OSTPRE – murtumanesto tutkimukseen (OSTPRE-FPS). OSTPRE-väestöstä poimituille 2928 naiselle tehtiin fyysiset toimintakykytestit ja murtumavalidaatio. OSTPRE-FPS oli kolme vuotta kestävä avoin randomoitu kontrolloitu tutkimus (vuosina 2003-2006) 3432 naiselle, jotka oli satunnaisesti valikoitu OSTPRE väestöotoksesta. Naiset satunnaistettiin kahteen samankokoiseen ryhmään. Interventioryhmä, johon kuului 1718 naista, käytti kolmen vuoden ajan kahdesti päivässä D-vitamiini ja kalsiumvalmistetta. Vuorokausiannos sisälsi 800 IU kolekalsiferolia ja 1000mg kalsiumkarbonaattia. Kontrolliryhmän 1714 naista eivät saaneet täydennysvalmistetta. Ennalta määritellylle 750 henkilön oheisotokselle tehtiin fyysiset toimintakykytestit ja luuntiheysmittaukset. Kaatumiset olivat henkilöiden itsensä ilmoittamia koko tutkimusväestössä (n=3432).

Naisilla, joilla oli reisiluun kaulan osteoporoosi, oli alentunut fyysinen toimintakyky verrattuna niihin, joiden luuntiheys oli normaali tai osteopeeninen. Kyky seistä yhdellä jalalla oli heillä alentunut -39% (p=0.001), puristusvoima -18% (p<0.001), alaraajan ojennusvoima -19% (p=0.007) ja kyky kyykistyä lattiaan asti -40% (p=0.004). Lisäksi naiset, joilla oli alentunut puristusvoima ja alhainen alaraajan ojennusvoima ja jotka eivät pystyneet seisomaan yhdellä jalalla kymmentä sekuntia eivätkä itse ilmoittamana kävelemään sataa metriä, saivat myöhemmin todennäköisemmin murtumia kuin muut. Näiden tulosten perusteella käden puristusvoimaa voidaan käyttää kliinisessä päätöksenteossa niiden henkilöiden tunnistamiseksi, jotka hyötyvät luuntiheysmittauksista, vaikka se ei yksin olekaan tarpeeksi tarkka osteoporoosin seulontaan. Lisäksi vaihdevuosien jälkeisiä murtumia voi ennakoida tutkimalla potilaan puristusvoimaa ja kykyä seistä yhdellä jalalla kymmenen sekunnin ajan sekä kysymällä, kykeneekö potilas kävelemään sata metriä. Voidaan olettaa, että huono fyysinen kunto lisää sekä alhaisen luuntiheyden yleisyyttä että kaatumisesta johtuvien murtumien riskiä.

OSTPRE-FPS tutkimus osoitti, että päivittäinen 800 IU D-vitamiinia ja 1000mg kalsiumia sisältävän täydennysvalmisteen käyttö voi vähentää toistuvien hoitoa vaatien kaatumisten riskiä 65-71 – vuotiaassa naisväestössä. Lisäksi D-vitamiini- ja kalsiumlisällä on myönteinen vaikutus naisten luuntiheyteen, vaikka kalsiumin saanti olikin tutkimukseen osallistuneilla lähtökohtaisesti riittävä. Kalsium- ja D-vitamiinilisällä ei ollut vakavia sivuvaikutuksia. Tutkimuksen perusteella voidaan suositella korkeampaa D-vitamiinin saantia 65-71 –vuotiaille naisille.

National Library of Medicine Classification: QU 173, QV 276, WE 180, WE 200, WA 288
Yleinen suomalainen asiasanasto: D-vitamiini, kaatuminen, kalsium, luu, luunmurtumat

ACKNOWLEDGEMENTS

This study was carried out in the Bone and Cartilage Research Center (BCRU), University of Eastern Finland (Kuopio) and Department of Orthopaedics, Traumatology and Hand Surgery, Kuopio University Hospital during the years 2004 to 2010.

I owe my deepest gratitude to my principal supervisor Professor Heikki Kröger, M.D., Ph.D., for warm support and encouragement during this work. During these years there has never been a moment that he could not make time to answer my questions. It has been privilege to write this thesis under his guidance.

I am thankful to my other supervisor Professor Jukka Jurvelin, Ph.D., for his support during the initial stages of this study.

I am deeply indebted to Docent Risto Honkanen, M.D., Ph.D., for his essential support. I admire his impressive scientific knowledge. I owe my sincere thanks to my co-workers and co-authors Professor Marjo Tuppurainen, M.D., Ph.D., Professor Esko Alhava, M.D., Ph.D., Kari Salovaara, M.D., Toni Rikkonen, M.Sc., Joonas Sirola, M.D., Ph.D., Docent Jari Arokoski, M.D., Ph.D., and Lorenzo Sandini, M.D. Without your support, this thesis would have never seen the daylight!

I am greatly indebted to Professor Ari Heinonen, Ph.D., Department of Health Sciences, University of Jyväskylä and Professor Timo Möttönen, M.D., Ph.D., Rheumatology Unit, Department of Internal Medicine, Turku University Hospital, for their valuable comments and criticism of the manuscript of this thesis. Their work considerably improved the final thesis.

I express my special thanks to the personnel of the BCRU, Ms. Seija Oinonen, Mrs. Sirkka Harle, Mrs. Marianna Elo and Mrs. Katri Karttunen. I want to thank Vesa Kiviniemi, Ph.Lic., for his kind advice and assistance with the statistical analyses. I thank Ewen MacDonald, D.Pharm., for his careful revision of the English language in the original publications and the thesis.

I dedicate my heartfelt thanks to my parents Marja-Leena and Jorma for their never-ending love and support in my life and to my sister Anna-Leena and my goddaughter Anni.

I express my deepest love to my girlfriend Anna, who has been standing by my side all these years – I love you so much!

I wish to express my gratitude to all friends, relatives and colleagues I have been honored to get know and to work with. You have been more valuable than you can imagine!

In appreciation of their financial support for this work, I would like to thank the North-Savo Regional Fund of the Finnish Cultural Foundation (Hulda Tossavainen Fund), Yrjö Jahnsson Foundation, Instrumentarium Foundation, Maud Kuistila Memorial Foundation, University of Kuopio and The Finnish Medical Foundation.

Last but not least, I want to thank all the women who took part in the OSTPRE and OSTPRE-FPS trials.

Jyväskylä, January 2011

Matti Kärkkäinen

LIST OF ORIGINAL PUBLICATIONS

The following doctoral thesis is based on four original articles, which are referred to their Roman numerals I-IV:

I Kärkkäinen M, Rikkonen T, Kröger H, Sirola J, Tuppurainen M, Salovaara K, Arokoski J, Jurvelin J, Honkanen R, Alhava E. Physical tests for patient selection for bone mineral density measurements in postmenopausal women. Bone. 2009 Apr;44(4):660-5.

II Kärkkäinen M, Rikkonen T, Kröger H, Sirola J, Tuppurainen M, Salovaara K, Arokoski J, Jurvelin J, Honkanen R, Alhava E. Association between functional capacity tests and fractures – 8 year prospective population based cohort study. Osteoporos Int. 2008 Aug;19(8):1203-10.

III Kärkkäinen M, Tuppurainen M, Salovaara K, Sandini L, Rikkonen T, Sirola J, Honkanen R, Arokoski J, Alhava E, Kröger H. Does daily vitamin D 800 IU and calcium 1000 mg supplementation decrease the risk of falling in ambulatory women aged 65 to 71 years? A three year randomized population-based trial (OSTPRE-FPS). Maturitas. 2010 Apr;65(4):359-65.

IV Kärkkäinen M, Tuppurainen M, Salovaara K, Sandini L, Rikkonen T, Sirola J, Honkanen R, Jurvelin J, Alhava E, Kröger H. Effect of calcium and vitamin D supplementation on bone mineral density in women aged 65 to 71 years – A three year randomized population-based trial (OSTPRE-FPS). Osteoporos Int. 2010 Dec;21(12):2047-55.

CONTENTS

ABBREVIATIONS

$1,25(OH)D_3$	Calcitriol
$25(OH)D_3$	Calcidiol
BMD	Areal bone mineral density
BMI	Body mass index
CI	Confidence interval
D_2	Ergocalciferol
D_3	Cholecalciferol
DXA	Dual x-ray absorptiometry
FRAX	Fracture risk assessment tool
FRMA	Falls requiring medical attention
GLM	General linear model
GS	Grip strength
HR	Hazard ratio
HT	Hormone therapy
OR	Odds ratio
PTH	Parathyroid hormone
RCT	Randomized controlled trial
ROC	Receiver operator characteristics
RR	Risk ratio
SOOF	Standing-on-one-foot test
UV-B	Ultraviolet-B radiation
VDR	Vitamin D receptor
WHO	World Health Organization

1 INTRODUCTION

In general, the fracture risk is highest among those who have osteoporotic BMD (Kröger et al. 1995, Pasco et al. 2006) but nonetheless over half of fragility fractures occur in subjects who do not have osteoporosis (Pasco et al. 2006, Sanders et al. 2006). Only in the population of age over 80 years does the majority of fractures occur in women with osteoporosis (Sanders et al. 2006). Accordingly, while the BMD is certainly one risk factor it is not the sole predictor for fractures. The importance of maintaining muscle strength to avoid bone loss has been emphasized (Sirola et al. 2006). In addition, poor balance and weak muscle strength of lower limbs have been associated with an increased incidence of falls (Tinetti et al. 1988, Nevitt et al. 1989, Dargent-Molina et al. 1996). Thus, the poor physical condition might predispose to both low BMD and a higher risk for fall-related fractures.

Each year, one third of individuals aged 65 or older will experience a fall (Campbell et al. 1981). Fall-related injuries require hospital admission in 42% of cases (Sattin et al. 1990) and 6–23% of those falling will suffer serious injuries (Nevitt et al. 1991, Tinetti et al. 1995). Vitamin D is now known to be of physiological importance beyond its effects on bone health and calcium homeostasis, for example there is evidence that it plays a role in the prevention of falling and it has been linked with beneficial properties in combatting a wide range of diseases.

Serum levels of 25-hydroxyvitamin D (25[OH]D) are directly related to BMD (Bischoff-

Ferrari et al. 2006). Low vitamin D status is prevalent in elderly women living in northern latitudes (Margiloff et al. 2001), but this deficiency can also be detected in young adults in northern Europe (Lamberg-Allardt et al. 2001) as well as in the housebound elderly living elsewhere (Gloth 3rd et al. 1995). Obviously this is a factor contributing to increased bone loss in the elderly.

The aims of the present studies were to determine whether relatively simple and clinically applicable physical capacity tests could provide clinically relevant information for detecting postmenopausal women who have osteoporosis and further could they help identify those subjects who will experience a fracture. In addition, there is still no definitive answer if vitamin D and calcium supplementation are effective as improving bone status, in particular, there are no population-based trials to resolve this issue. Furthermore, there are no population-based trials concerning the effects of vitamin D and calcium supplementation on fall prevention.

2 REVIEW OF THE LITERATURE

2.1 Osteoporosis and bone strength

Bone strength depends not only on the material properties but also on size, shape and three-dimensional architecture of the bone (Ahlborg et al. 2003). Bone has the ability to modify its composition and structure to accommodate prevailing loads (Seeman et al. 2007). Areal bone mineral density (BMD) is a powerful determinant of bone strength (Kanis et al. 2008). The National Institutes of Health (NIH) has defined osteoporosis as a disease of increased skeleton fragility accompanied by low BMD and microarchitectural deterioration (National Institutes of Health 2001). Trabecular bone loss has been reported to begin in young adulthood and one can detect substantial cortical bone loss in midlife (Riggs et al. 2008). An accelerated trabecular bone loss occurs in the perimenopause (Riggs et al. 2004). It has been shown that in the upper end of femur, bone loss occurs uniformly throughout the femoral neck, leading to an overall decrease in femoral bone mass and trabecular thickness (Tanck et al. 2009). Secondary causes for osteoporosis, such as certain systemic diseases (Poole et al. 2002) and medications (van Staa et al. 2002) as well as osteomalacia and malignancy, induce both bone loss and fractures.

The clinical relevance of osteoporosis lies in the subsequent risk of fractures. Recovery from hip fracture is slow and often incomplete, with many patients being permanently institutionalised. Vertebral fracture, another common osteoporotic fracture, causes acute pain and loss of function (Kanis et al. 2008). Premature mortality following hip and

vertebral fractures is well documented (Center et al. 1999, Cauley et al. 2000).

2.2 Diagnosis and screening of osteoporosis

Bone mineral density (BMD) is determined by both the peak bone density achieved during the growth period up to skeletal maturity and the subsequent bone loss related to age and menopause (Hui et al. 1990). Up to 70% of the variation of bone density is determined by heredity (Vicente-Rodriguez et al. 2007). Several gene sequence variants are associated with BMD and low trauma fractures (Styrkarsdottir et al. 2008). The diagnosis of osteoporosis is generally based on the assessment of BMD at the spine or the femoral neck by dual energy X-ray absorptiometry (DXA). The results obtained are interpreted according to the WHO definition of osteoporosis (i.e. a value for BMD 2.5 or more below the young adult mean). Measurements at the femoral neck have the highest prediction for hip fracture risk (Marshall et al. 1996, Johnell et al. 2005) but BMD measurements cannot identify subjects who will subsequently experience a fracture (Marshall et al. 1996). Femoral measurement has also of highest clinical relevance due to the morbidity associated with hip fracture (Melton III 2003).

Non-selective, population-based screening for osteoporosis with DXA is not recommended (Kanis et al. 2005). Several decision rules, based on clinical criteria, for BMD referrals have been developed (Michaëlsson et a. 1996, Lydick et al. 1998, Cadarette et al. 2000, Weinstein et al. 2000). These decision rules are not meant to replace diagnostic tests, but to help to identify high-risk individuals that may benefit from

BMD testing (McGinn et al. 2000). These risk assessment tools have been evaluated (Cadarette et al. 2000, Gourlay et al. 2008) but their suitability for case-finding approaches is controversial. However, BMD is only one of many factors that independently influences the fracture risk. WHO have introduced the fracture risk assessment tool FRAX^{TM} that has been developed based on the use of clinical risk factors with or without BMD (Kanis et al. 2008). Its validation to predict hip fractures has shown that there is a strong positive correlation between predicted and observed ratios (Leslie et al. 2010).

Osteoporosis and osteoporotic fractures are strongly associated with age (Boonen et al. 2008). Indeed, elderly patients with a prevalent fragility fracture have been suggested to be in need of osteoporosis treatment, regardless of their BMD (Boonen et al. 2008). However, repeat low-trauma fractures constitute nearly one half of the fracture burden in women and this has been found to be independent of BMD (Langsetmo et al. 2009). Accordingly, the proposal to generalise osteoporotic treatment not only to the population of osteopenic but also with normal BMD is difficult to justify since only a few pharmacological interventions have any effect to prevent nonvertebral fractures (Chapurlat et al. 2006) and their antifracture efficacy could be even more questionable in non-osteoporotic subjects. The pharmacological interventions that are currently in use effect primarily on vertebral fracture prevention (Chapurlat et al. 2006). Other osteoporosis drugs have been introduced and these agents have a more anabolic effect on bone (Neer et al. 2001, Cummings et al. 2009). Currently the following drugs for osteoporosis treatment are available on prescription in Finland: alendronate, etidronate,

risedronate, ibandronate, tsoledronate, estrogen, raloxifene, calcitonin, testosterone, strontium ranelate, teriparatide, parathyroid hormone (PTH) and denosumab.

2.3 Falls

One third of individuals aged 65 and older fall at least once each year and about half of these fall twice or more (Tinetti et al. 1988, Nevitt et al. 1989). The consequences of falling are severe; 3-6% of falls lead to a fracture (Tinetti et al. 1988, Stel et al. 2004), 68% to a physical injury (Stel et al. 2004) and 12% cause a serious injury (Tinetti et al. 1995). Falls are an important external cause for fractures to distal radius (Vogt et al. 2002), proximal humerus (Kristiansen et al. 1987) and hip (Hayes et al. 1993). In fact, fall impact directly at the greater trochanter of the proximal femur increases the relative risk of hip fracture by as much as 30-fold (Hayes et al. 1993, Nevitt et al. 1993, Greenspan et al. 1998). However, only 25% of vertebral fractures result from falls (Cooper et al. 1992).

Non-syncopal falls have complex and diverse causes. Maintaining an upright posture requires sensory input from the visual, tactile, proprioceptive and vestibular systems, central processing and a well coordinated motor response (Lord et al. 1994, Tinetti et al. 1997). Further, ankle flexibility, plantar tactile sensation and foot muscle strength play major roles in balance in older individuals (Menz et al. 2005). The use of certain medications increases the likelihood of falling in elderly subjects (Woolcott et al. 2009). Falling is multifactorial caused by intrinsic and extrinsic risk factors, usually a

combination of factors (Graafmans et al. 1996). In addition, the fall mechanism leading to hip or wrist fracture is different from that in non-injurious falls (Nevitt et al. 1993). Patients who fall have impaired functional performance and psychomotor function (Dhesi et al. 2002). Falls that happen indoors have been associated with a subsequent functional decline (Manty et al. 2009). Furthermore, balance, gait, neuromuscular and cognitive impairment have been associated with a risk of experiencing a serious injury during a fall (Nevitt et al. 1991, Tinetti et al. 1995). In addition, subjects who have fallen and have early signs of mobility decline (Mänty et al. 2010), gait or balance problems (Ganz et al. 2007) or impaired physical and cognitive function (Formiga et al. 2008) are at an especially high risk of suffering subsequent falls.

Earlier studies on multifactorial fall prevention in the elderly have shown both the positive (Tinetti et al. 2003, Kannus et a. 2005) and conflicting results (Gates et al. 2008, de Vries et al. 2010). However, several single-intervention strategies for fall prevention have been proven to be beneficial. Strength and balance training can reduce the risk of both non-injurious and injurious falls (Campbell et al. 1997, Robertson et al. 2001, Robertson et al. 2002, Day et al. 2002, Tinetti et al. 2003, Chang et al. 2004). However, the risk of falling may be increased in both the most physically active as well as the inactive persons (Moayyeri 2008), though this proposal could not be confirmed in a recent study (Peeters et al. 2010). On the other hand, walking and leisure-time physical activity have been shown to reduce the risk of subsequent hip fractures (Feskanich et al. 2002) and recurrent falling (Peeters et al. 2010). It has been postulated that recurrent fallers especially might benefit from prevention based on

mobility improvement (Graafmans et al. 1996). Vitamin D and calcium have been shown to reduce the risk of falls in ambulatory and institutionalized elderly subjects (Callagher et al. 2001, Bischoff-Ferrari et al. 2004, Bischoff-Ferrari et al. 2004, Harwood et al. 2004, Larsen et al. 2005). In addition, withdrawal of psychotropic drugs (Campbell et al. 1999), cataract surgery (Harwood et al. 2005) and home hazard assessment and modification (Gillespie 2009) have all been shown to reduce the risk of falling in the elderly.

2.4 Fractures

In women over the age of 60 years, the risk of fracture has been shown increase 6% per year of age (Pasco et al. 2006). Osteoporotic fractures have generally been defined as fractures that occur following relatively low trauma, such as a fall from standing height or less (Center et al. 2007). Fractures of the vertebrae, proximal femur and distal forearm have been regarded as the traditional osteoporotic fractures (Cummings et al. 2002). Hip BMD has been associated with almost all types of fractures, an association that is stronger than with spinal or peripheral BMD (Stone et al. 2003). Low BMD has been associated with both the low and high trauma fractures (Mackey et al. 2007). However, less than one-half of fractures have been reported to be attributable to osteoporosis (Stone et al. 2003). The 5-year age-standardised absolute fracture risk has been shown to rise from 7% in persons who have normal BMD up to 47% in individuals who suffer osteoporosis and prevalent fracture (Pasco et al. 2006). In general, the fracture risk is highest among those who have osteoporotic BMD (Kröger et

al. 1995, Pasco et al. 2006) but most fragility fractures occur in subjects who are not suffering from osteoporosis (Pasco et al. 2006, Sanders et al. 2006). Postmenopausal women with the highest physical activity level also have a moderately higher wrist fracture risk despite their slower femoral bone loss (Rikkonen et al. 2010). In fact, risk of falling has been more closely associated with limb fracture risk than with BMD (Kaptoge et al. 2005). It has been postulated that the underlying mechanism of early premenopausal non-wrist fractures is an increased propensity to trauma rather than a low BMD value (Honkanen et al. 1997). In addition, neuromuscular impairment has been associated with both hip (Dargent-Molina et al. 1996) and proximal humerus fractures (Kelsey et al. 1992). The important risk factors for hip and wrist fractures apparently relate to bone strength and falls (Nevitt et al. 1993, McClung et al. 2001).

The burden of osteporotic fractures relates to the morbidity and associated mortality (Cooper 1997, Center et al. 1999). In the first year following a hip fracture, 10-20% of patients die from its complications (Cummings et al. 2002). In contrast, distal forearm fractures do not elevate mortality rates (Cooper et al. 1993). The events surrounding the fracture are at least part of the cause of the excess mortality (Bliuc et al. 2009). The underlying health of the patient is closely related to mortality (Tosteson et al. 2007). In all, 50% of all low-trauma fractures have been reported to be nonhip and nonvertebral fractures, and to be associated with more than 40% of all deaths (Bliuc et al. 2009). If one focuses exclusively on hip fractures then there is the risk of underestimating the contribution of osteoporosis and the need for its management (Delmas et al. 2007). It has been concluded in meta-analyses that a previous fracture increases the risk for a

subsequent fracture by 2-fold, and a prior vertebral fracture increases the risk for subsequent vertebral fracture by 4-fold (Klotzbuecher et al. 2000, Kanis et al. 2004). A previous wrist fracture increases the risk for subsequent wrist fracture by 1.6-fold (Honkanen et al. 2000). It has been claimed that previous fragility fractures predict future ones (Lauritzen et al. 1993, Honkanen et al. 1997). In fact, half of women will experience another fracture in the following 10 years (Center et al. 2007). A subsequent fracture increases mortality risk by 3- to 4-fold (Bliuc 2009). In addition, the subsequent fracture might be a hip or other major fracture even though the initial fracture was only a minor one (Center et al. 2007).

2.5 Physical capacity

Regular physical activity is important in preserving acquired peak bone mass during puberty (Rautava et al. 2007). Resistance training affects positively the maintenance of regional femoral BMD (Ryan et al. 1998). In particular, the weight-bearing bones of the lower extremities benefit from long-term physical activity (Rikkonen et al. 2006). Muscle strength has been reported to account for 15-20% of the total variance in BMD in young women (Snow-Harter et al. 1990) and as much as 30% of the variability of total skeletal BMD can be explained by lean mass and the amount of physical exercise (Valdimarsson et al. 1999). Neuromuscular performance has been shown to independently associate with bone strength (Rantalainen et al. 2010). The association of grip strength with spinal (Sirola et al. 2005), femoral (Cauley et al. 2005) and forearm BMD (Bevier et al. 1989) might point to a systemic relationship between muscle

strength and BMD. In addition, grip strength is associated with bone loss (Sirola et al. 2005) and future fractures (Sirola et al. 2006). However, changes in grip strength have not been found to be associated with changes in the radial BMD (Wang et al. 2004).

Back extensor muscle strength and lumbar spine mobility have been found to be associated with quality of life in postmenopausal osteoporotic patients (Miyakoshi et al. 2007). In a subsequent trial, back extensor strength exercise was found to improve the quality of life (Hongo et al. 2007). Poor lower-extremity performance is strongly predictive of future disability, hospitalization, and premature mortality (Guralnik et al. 1995). In addition, hand grip strength has been found to predict functional limitations and disability in men (Rantanen et al. 1999) and frailty in older women (Syddall et al. 2003).

Physical activity and muscle strength have been shown to predict the severity of disability (Rantanen et al. 1999). Loss of strength in lower limb muscles (Macrae et al. 1990) and ankle weakness are important factors contributing to poor balance (Whipple et al. 1987). The get-up and go test has been reported to be a reliable way to assess the balance of elderly patients (Mathias et al. 1986) and basic functional mobility (Podsiadlo et al. 1991). In addition, a poor tandem walk predicted recurrent falling in home-dwelling elderly fallers (Nevitt et al. 1989). Physical inactivity has been associated with increased risk for ankle and wrist fractures (Honkanen et al. 1998). In males, a high degree of leisure-time physical activity has been reported to be able to protect against hip fracture (Trimpou et al. 2010). It has been claimed that everyday physical activities

such as household work, walking, and gardening may play an important role in maintaining strength in older people at an adequate level to permit independent living (Rantanen et al. 1997).

2.6 Role of vitamin D and calcium

2.6.1 Vitamin D functions and considerations for sufficient daily dose

Vitamin D_2 (ergocalciferol) and vitamin D_3 (cholecalciferol) are the two forms of vitamin D. In the skin, with the effect of ultraviolet B (UVB) radiation, 7-dehydrocholesterol is photoconverted to previtamin D_3, which is further converted to vitamin D_3 (cholecalciferol). In the serum, vitamin D_3 is bound to a vitamin D binding protein and transported to the liver, where it is hydroxylated to 25(OH)D_3. In the kidneys, 25(OH)D_3 is further metabolized to 1,25-dihydroxyvitamin D_3 [1,25(OH)D_3] and this is a biologically active form of vitamin D_3 (Dusso et al. 1998). It is known that the colon, prostate, breast, brain, β-islet cells, vascular smooth muscle cells and macrophages are able to produce 1,25(OH)D_3 (Schwartz et al. 1998, Cross et al. 2001, Holick 2007). Vitamin D_3 can obtained from oily fish, fortified dairy products or supplements. Vitamin D_3 has been shown to increase serum 25(OH)D level more efficiently than vitamin D_2 (Trang et al. 1998, Armas et al. 2004). The serum 25(OH)D concentration reflects both endogenous production and intake of vitamin D, and therefore it is clinically useful (Holick 2007).

Earlier vitamin D sufficiency was defined as the level of 25(OH)D sufficient to prevent rickets in children and osteomalacia in adults (Bikle et al. 2008). Currently, there is no clear definition of optimal vitamin D status. However, studies of the relationship between 25(OH)D and BMD (Bischoff-Ferrari et al. 2004), fractures (Bischoff-Ferrari et al. 2005), lower extremity function (Bischoff-Ferrari et al. 2004), periodontal disease (Krall et al. 2001) and colorectal cancer (Bischoff-Ferrari et al. 2006) have shown that a serum level of 75nmol/l or greater can be considered to be a sufficient vitamin D level (Dawson-Hughes et al. 2005, Holick 2007). High rates of vitamin D deficiency have been reported in apparently healthy children (Lehtonen-Veromaa et al. 1999, Holick 2007), young adults (Gordon et al. 2004, Sullivan et al. 2005) and the elderly (Holick 2007). Young adults have been shown to have an equal to greater risk of vitamin D insufficiency than older people (Tangpricha et al. 2002). Further, food fortification with vitamin D has been reported to result in only a minor effect in adolescent Finnish females (Lehtonen-Veromaa et al. 2008).

Naturally, the vitamin D intake needed to raise the level to 75nmol/l is dependent on basal 25(OH)D concentrations (Bischoff-Ferrari 2007) and on calcium status (Heaney 2008). One study recommended at least 1000IU of vitamin D daily and an adequate amount of sun exposure for everyone to combat against vitamin D deficiency (Holick 2008). A vitamin D_3 dose of 3,800IU for those with serum levels above 55nmol/l of 25(OH)D and a dose of 5,000IU for those below that threshold have been proposed (Aloia et al. 2008). Another study showed that administration of vitamin D_3 of 400IU for eight weeks would elevate the serum 25(OH)D by 11nmol/l (Barger-Lux et al. 1998),

while 4,000IU daily are needed to raise 88% of healthy young adults to at least 75nmol/l (Vieth et al. 2001). The recent Institute of Medicine recommendations for daily dietary intake of vitamin D 600-800IU for adults are purely based on bone benefits (Institute of Medicine 2010) and they are very low compared to Bischoff-Ferrari et al. recommendations of oral doses of 1800 to 4000IU vitamin D daily which are based on other health benefits and higher necessary serum 25(OH)D levels (Bischoff-Ferrari et al. 2010).

It has been estimated that the body can use up to 5,000IU of vitamin D daily (Heaney et al. 2003). In addition, vitamin D supplementation has not been found to be toxic at daily doses below 10,000IU (Hathcock et al. 2007). Hypercalcaemia has been suggested to be a risk associated with synthetic vitamin D analogues or from calcium components of supplements combining calcium and vitamin D (Rhein 2009, Quinton et al. 2009). Large doses have been used in vitamin D deficient subjects and it has been shown that a loading dose of 500,000IU and then 50,000IU monthly are a safe way to normalize 25(OH)D levels (Bacon et al. 2009). A recent study reported that for subjects with severe vitamin D deficiency, a single oral dose of 300,000IU raises mean 25(OH)D levels above 75nmol/l at three months (von Restorff et al. 2009). It has been shown that mean serum calcium levels are not related to oral vitamin D at doses up to 100 000IU per day or achieved serum 25(OH)D up to 643nmol/l (Bischoff-Ferrari et al. 2010).

Several reasons have been proposed to explain the high prevalence of vitamin D deficiency. Both the capacity of the skin to synthesise the previtamin (MacLaughlin et al.

1985) as well as the expression of the vitamin D receptor decreases with age (Bischoff-Ferrari et al. 2004, Venning 2005). In addition, less time spent outdoors (van der Wielen et al. 1995) and covering the skin with clothes (El-Hajj Fuleihan et al. 2001) lead to decreased exposure to UVB radiation. Seasonal fluctuations in $25(OH)D_3$ concentrations have been observed (Lawson et al. 1979, Dawson-Hughes et al. 1997). Low 25(OH) levels in winter have been associated with increased PTH levels, bone resorption, the proportion of falls resulting in fracture and the frequency of hip and wrist fracture (Pasco et al. 2004).

2.6.2 Functions of calcium

Calcium is important for numerous cellular functions including cell division, cell adhesion and plasma membrane integrity, protein secretion, muscle contraction, neuronal excitability, glycogen metabolism and coagulation (Favus et al. 2008). In addition, it is essential for bone formation (Dawson-Hughes 2008).

The concentrations of total calcium in normal serum generally range between 2.12 and 2.62nmol/l. The maintenance of the serum calcium is regulated by ion transport in the kidney, intestinal tract and bone. Low $1,25(OH)D_3$ levels and PTH stimulate the osteoclasts to resorb bone, which leads to calcium release into the extracellular fluid. Though several other hormones and clinical conditions can stimulate distal renal tubular calcium reabsorption, PTH is an important hormone in this respect (Bushinsky et al. 1998, Favus et al. 2008). $1,25(OH)D_3$ enhances intestinal calcium absorption

(Bushinsky et al. 1998) and the malabsorption of calcium only occurs at very low levels of 25(OH)D (Need et al. 2008). However, an elevation in PTH levels and calcium malabsorption can evoke a major increase in bone turnover (Need et al. 2008). Therefore, vitamin D insufficiency will have direct effects on bone histology (Need et al. 2008).

Vitamin D has been reported to have a calcium sparing effect (Steingrimsdottir et al. 2005). Calcium intake greater than 800mg daily might be unnecessary to adequate calcium metabolism if vitamin D status is ensured (Steingrimsdottir et al. 2005). This could be explained by improved calcium absorption at higher 25(OH)D levels (Steingrimsdottir et al. 2005). Indeed, vitamin D sufficiency has been shown to be more important than a high calcium intake in the maintenance of adequate levels of serum PTH (Steingrimsdottir et al. 2005). Interestingly, vitamin D status and its associated benefits are also believed to be dependent on sufficient calcium intake (Heaney 2008). The Institute of Medicine recently recommended a daily dietary intake of calcium 1000-1200mg for adults (Institute of medicine 2010). However, the recent American Society for Bone and Mineral research statement concluded that beneficial effects of calcium are achieved at relatively low doses (American Society for Bone and Mineral Research 2010). There are few findings indicating that calcium supplementation without co-administered vitamin D would be associated with adverse cardiovascular events (Pentti et al. 2009, Bolland et al. 2010). In addition, elderly individuals and subjects with renal impairment have been reported to be at a higher risk of suffering cardiovascular

problems if they are taking calcium supplements (American Society for Bone and Mineral Research 2010).

Hypercalcemia occurs generally when the influx of calcium from the bone or intestine exceeds the renal calcium excretory capacity (Bushinsky et al. 1998). Growth, pregnancy, primary hyperparathyroidism and sarcoidosis increase intestinal calcium absorption (Favus et al. 2008). Hypocalcemia occurs when calcium loss from the extracellular fluid is greater than can be replaced by absorption from the intestine or bone (Bushinsky et al. 1998). Vitamin D deficiency, chronic renal insufficiency, aging and excess glucocorticoids decrease calcium absorption (Favus et al. 2008).

2.6.3 Vitamin D is associated with muscle function

Vitamin D receptor is present in skeletal muscle (Simpson et al. 1985). Vitamin D has been reported to exert both genetic and non-genetic effects on muscle function (Bischoff-Ferrari et al. 2004, Campbell et al. 2006). First, 1,25(OH)D_3 binds to a nuclear vitamin D receptor (VDR). This leads to direct gene transcription and de novo protein synthesis (Boland et al. 1986). Second, the binding of 1,25(OH)D_3 on VDR activates certain second-messenger pathways, resulting in enhanced calcium uptake through calcium channels in the cell membrane (Campbell et al. 2006).

Recent findings have been shown that vitamin D levels are associated with muscle power and force in adolescent girls (Ward et al. 2009). Previously higher 25(OH)D

levels have been associated with improved lower extremity function in the elderly (Bischoff-Ferrari et al. 2004) and consistently low levels were linked with reduced physical performance (Wicherts et al. 2007). Serum levels of 25(OH)D of 80 - 100nmol/l are believed to be most advantageous for lower extremity strength (Bischoff-Ferrari et al. 2004, Wicherts et al. 2007). Doses of 400IU of vitamin D and 1000mg of calcium carbonate for elderly women have been found to be ineffective in combatting against a decline of physical functioning (Brunner et al. 2008) since this dose is not sufficient to elevate vitamin D to the desirable level, as discussed earlier. However, vitamin D 400IU and calcium 800mg have been reported to improve gait speed and body sway and training to improve muscle strength in vitamin D deficient subjects (Bunout et al. 2006). Whereas, doses of vitamin D 800IU and calcium 1000mg have been reported to improve muscle function in community-dwelling older individuals (Bischoff-Ferrari et al. 2003, Pfeifer et al. 2009).

2.6.4 How does vitamin D prevent falling?

Frailty in older adults has been shown to increase with age and it has been associated with incident falls (Fried et al. 2001). Most falls in the elderly result from trips or slips, when the impaired balance of an elderly individual prevents swift corrective action (Campbell et al. 2005). During sudden movements, the fast and strong type II muscle fibers react first to avoid falling (Pfeifer et al. 2002). In osteomalacic patients, the type II fibers are believed to become atrophied (Yoshikawa et al. 1979), and myopathy has been associated with osteomalacia (Skaria et al. 1975). Low vitamin D status causes

muscle weakness, which may lead to further falls (Pun et al. 1990). The fact that primarily type II fibers are affected might explain the falling tendency of vitamin D deficient subjects (Pfeifer et al. 2002). In fact, vitamin D_2 supplementation has been shown to increase the diameter of fast twitch type II muscle fibers (Sato et al. 2005). To be exact, vitamin D deficiency affects predominantly the weight-bearing muscles of the lower limb (Mingrone et al. 1999, Campbell & Allain 2006). Consistently, improved 25(OH)D status has been associated with better lower-extremity function in the elderly (Bischoff-Ferrari et al. 2004) and patients with low 25(OH)D levels have been shown to have impaired functional performance, psychomotor function, muscle strength and increased falling tendency (Dhesi et al. 2002). Vitamin D has been reported to improve neuromuscular function (Glerup et al. 2000, Bischoff et al. 2003, Bischoff-Ferrari et al. 2004) and balance (Pfeifer et al. 2000). The treatment effect of vitamin D and calcium supplementation has been reported to be attributable to improvements in postural and dynamic balance (Bischoff-Ferrari et al. 2006) and reduced body sway (Pfeifer et al. 2000). In one other trial, once a week vitamin D supplementation did not reduce medio-lateral sway (Lips et al. 2010). It is noteworthy that severe hypovitaminosis D myopathy may appear before there are any signs of decreased bone mass (Glerup et al. 2000). In addition to the myopathy, vitamin D deficiency has been shown to induce neuropathy (Skaria et al. 1975). Vitamin D insufficiency has further been associated with abnormal development and functioning on the central nervous system (Tuohimaa et al. 2009). This could have some influence on coordinative muscle function.

The subsequent elevation in the PTH level is a consequence of low vitamin D intake. A

recent study revealed that PTH has a lesser effect upon muscle function (Ward et al. 2009) and this has been supported by earlier studies (Bischoff et al. 2003, Sambrook et al. 2004). However, there are also conflicting findings of the independent role of PTH in proximal myopathy (Chou et al. 1999).

Low 25(OH)D level has been associated with increased fall incidence (Dhesi et al. 2002, Bischoff et al. 2003). Vitamin D supplementation has been shown to reduce the risk of falling by 14-22% in ambulatory and institutionalized elderly (Bischoff-Ferrari et al. 2004, Kalyani et al. 2010). In a recent meta-analysis, supplemental vitamin D in a daily dose of 700-1000IU decreased the risk of falling by 19% among older subjects (Bischoff-Ferrari et al. 2009). The institutionalized elderly women who experience recurrent falls (Bishoff-Ferrari et al. 2003) and ambulatory less physically active women (Bischoff-Ferrari et al. 2006) have been suggested to benefit most from supplementation. Combined vitamin D and calcium supplementation have been shown to be superior to calcium alone in reducing the number of falls (Pfeifer et al. 2009). In addition, adequate calcium intake has been suggested to be necessary to achieve optimal vitamin D action (Kalyani et al. 2010). However, alphacalcidol has been shown to reduce number of elderly fallers when the minimum daily calcium intake was only 500mg (Dukas et al. 2004).

2.6.5 Effect of vitamin D and calcium on bone loss

Calcium and vitamin D are needed for bone growth in children and adolescents as well

as to decrease bone loss in adults and the elderly (Dawson-Hughes 2008). Bone loss has been suggested to be different in the axial and peripheral skeleton (Hansen et al. 1995). Hip bone loss has been reported to increase during the last years of life (Greenspan et al. 1994, Ensrud et al. 1995).

A close relationship between vitamin D status and BMD has been shown (Mezquita-Raya et al. 2001, Bischoff-Ferrari et al. 2004). Pubertal girls with hypovitaminosis D have been shown to have a risk of not achieving maximum peak bone mass (Lehtonen-Veromaa et al. 2002). A recent study indicated that serum 25(OH)D is a more important predictor of hip BMD than calcium intake (Biscohoff-Ferrari et al. 2009). Resolution of vitamin D insufficiency seems to result in a rapid increase in BMD (Adams et al. 1999). However, conflicting results about vitamin D status and bone health have also been reported (Garnero et al. 2007). One study reported a positive effect of vitamin D and calcium fortified dairy products on BMD though no effect was found with calcium alone (Moschonic et al. 2006). In early postmenopausal women with adequate vitamin D levels, calcium alone has been shown to be as effective as vitamin D (Cooper et al. 2003).

Healthy postmenopausal and elderly women who have a low calcium intake have been reported to benefit from suffering bone loss if they are given calcium supplementation (Dawson-Hughes et al. 1990, Elders et al. 1991, Chevalley et al. 1994, Storm et al. 1998). However, calcium supplementation has been reported to have only a minor effect against cortical bone loss but no effect on trabecular bone loss (Riis et al. 1987).

The minor effect of calcium supplementation on bone loss has been later confirmed in the elderly (Riggs et al. 1998), on bone density in healthy children (Winzenberg et al. 2006) and prepubertal girls (Bonjour et al. 1997). However, calcium has been also claimed to result in a reduction of bone loss and turnover in healthy postmenopausal women (Reid et al. 2006).

In a meta-analysis, calcium alone and calcium with vitamin D_3 have been associated with reduced bone loss at the hip and spine (Tang et al. 2007). In a more recent review, it was speculated that calcitriol might have been able to reduce or even reverse bone loss in postmenopausal women (Peppone et al. 2010). In another study, the effect was non-significant with 400IU of vitamin D_3 and 1000mg of calcium at the spine as well as in the total body (Jackson et al. 2006). However, a positive result with 560IU of vitamin D_3 and 1000mg of calcium on spinal BMD has also been reported (Baeksgaard et al. 1998). Dawson-Hughes et al. claimed that 500mg of calcium and 700IU of vitamin D_3 supplementation had no effect on proximal femur of postmenopausal women who were living in the community (Dawson-Hughes et al. 1997). However, an increase of femoral BMD during a shorter 18 months follow-up has also been reported with 1200mg of calcium and 800IU of vitamin D_3 (Chapuy et al. 1992) and the positive effects of 400IU vitamin D_3 supplementation on femoral BMD have been confirmed in the elderly women (Ooms et al. 1995). Chapuy et al. detected a lower rate of annual bone loss at the proximal femur in 1200mg of calcium and 800IU of vitamin D_3 supplemented ambulatory institutionalized women (Chapuy et al. 2002) but 1000IU vitamin D_2 had no effect on bone (Zhu et al. 2008). Another study showed that in patients with a low-energy

fracture, 1400IU of vitamin D_3 and 3000mg of calcium could decrease bone loss (Hitz et al. 2007). These conflicting findings could be partly explained by variations in dosages and different intervals between dosing (Chel et al. 2008). In addition, different forms of vitamin D (Richy et al. 2005) and the heterogeneity of study populations, including variations in baseline nutritional calcium intake and estrogen status, might have confounded the results.

In postmenopausal women, it has been reported that there is reduced loss of total body BMD after supplementation (Dawson-Hughes et al. 1997) but some studies have reported negative results in younger study populations (Hunter et al. 2000, Patel et al. 2001). The effect against bone loss in total body (Dawson-Hughes et al. 1997) might be explained by effect of calcium and vitamin D on endocortical and cortical bone loss (Nordin et al. 1985, Riis et al. 1987, Daly et al. 2006) since the skeleton has a higher proportion of cortical than trabecular bone.

Vitamin D supplementation has been shown to be effective against wintertime bone loss in healthy postmenopausal women (Dawson-Hughes et al. 1991). The effect of vitamin D and calcium supplementation against seasonal bone loss has been reported also in healthy adults (Meier et al. 2004).

2.6.6 Vitamin D and calcium on fracture prevention

The muscle weakness, which is associated with vitamin D deficiency, (Glerup et al.

2000) may increase fracture risk by causing greater susceptibility to falls (Bischoff et al. 2003, Visser et al. 2003, Sharkey et al. 2003). In addition, the protective effect of vitamin D on fractures has been attributed to the benefits of vitamin D on calcium homeostasis and BMD (Ooms et al. 1995, Dawson-Hughes et al. 1997). Furthermore, the decreased fracture incidence seen after vitamin D treatment has been proposed to be due to improvements in bone quality, which are not measurable by standard DXA, and/or in the ability of the neuromuscular system to prevent falls (Heikinheimo et al. 1996). The reason for decreased fracture incidence with vitamin D and calcium supplementation is still a matter of intense debate (Bischoff-Ferrari et al. 2009, The Dipart Group 2010).

Low 25(OH)D levels have been associated with a higher risk for hip fracture (Cauley et al. 2008) and osteoporotic fractures (van Schoor et al. 2008). A meta-analysis revealed that vitamin D supplementation could lower the risk of hip fracture by 26% and any nonvertebral fracture by 23% (Bischoff-Ferrari et al. 2005). Daily dose of more than 400IU of vitamin D reduced nonvertebral fractures in community-dwelling subjects by 29% and in institutionalized elderly by 15% according to a recent meta-analysis (Bischoff-Ferrari et al. 2009). And it was concluded that nonvertebral fracture prevention with vitamin D was dose dependent (Bischoff-Ferrari HA et al. 2009). However, some trials have not found any effect on fractures of vitamin D, or vitamin D combined with calcium (Grant et al. 2005, Porthouse et al. 2005, Jackson et al. 2006). The negative results have been explained in part by poor compliance and the low dose of vitamin D supplement (Bischoff-Ferrari 2007). In fact, in a trial with better compliance, the hip

fracture risk was reduced by 29% (Jackson et al. 2006). A recent meta-analysis found that calcium and vitamin D together, but not vitamin D alone could reduce hip fractures (The Dipart Group 2010). However, that study was not adjusted for compliance which may explain the contrasting conclusions between it and the Bischoff-Ferrari et al. meta-analysis.

Several factors have been identified to improve adherence in clinical trials (Brunner et al. 2009). Intermittent higher doses of vitamin D applied by intra-muscular injection or orally may increase adherence (Bischoff-Ferrari 2007). However, daily administration of 800IU of vitamin D_3 has been reported to be more efficient than 100 000IU every four months in its ability to elevate the serum 25(OH)D concentration (Pekkarinen et al. 2010). A dose of 100 000IU of vitamin D orally every four months over five years was effective at preventing hip, wrist or forearm and vertebral fractures, an effect was achieved without evoking any adverse effects (Trivedi et al. 2003). Furthermore, annual intramuscular injections have been shown to prevent fractures in the upper limb (Heikinheimo et al. 1992). Surprisingly, it was reported that a single annual dose of 500 000IU of cholecalciferol resulted in an increased risk of falls and fractures in the first three months following dosing (Sanders et al. 2010).

It has been concluded that increased calcium intake alone is not able to reduce the risk of hip fracture (Bischoff-Ferrari et al. 2007, Reid et al. 2006, Reid et al. 2008), although conflicting results also exist (Tang et al. 2007).

2.6.7 Vitamin D is associated with a variety of diseases

The mechanism of 1,25(OH)D_3 actions makes it essentially a hormone (Fraser 1995, Lee et al. 2008) and in fact 1,25(OH)D_3 has been shown to directly or indirectly regulate over 200 genes (Holick 2007).

Earlier findings have associated vitamin D deficiency with a 30-50% increased risk of colorectal, breast and prostate cancer, (Garland et al. 1985, Ahonen et al. 2000, Grant 2002, Giovannucci et al. 2006, Jenab et al. 2010), and depression (Gloth 3rd et al. 1999) and pre-eclampsia (Bodnar et al. 2007). However, serum 25(OH)D levels do not seem to be associated with the risk of prostate cancer (Travis et al. 2009). A daily dose of at least 400IU of vitamin D was reported to reduce the risk of oesophagus and pancreas cancer and non-Hodgkin lymphoma (Giovannucci et al. 2006) and also over 40% reductions in the risks of multiple sclerosis (Munger et al. 2004) and rheumatoid arthritis (Merlino et al. 2004). Subjects who are living at higher latitudes and are at risk of vitamin D deficiency have increased risk of type I diabetes (Stene et al. 2000), multiple sclerosis (Embry et al. 2000, Ponsonby et al. 2002), hypertension (Rostand 1997) and schizophrenia (McGrath et al. 2002). Low 25(OH)D levels have been associated with a cognitive decline in elderly population (Llevellyn et al. 2010). Recent findings have pointed to a role for vitamin D in innate immunity (Walker et al. 2009) and preventing upper respiratory tract infections (Ginde et al. 2009). Furthermore, vitamin D deficiency has been associated with increased risk of myocardial infarction (Scragg et al. 2007, Wang et al. 2008), ischemic stroke and heart failure (Scragg et al. 2007),

cardiovascular (Dobnig et al. 2008), cerebrovascular death (Kilkkinen et al. 2009) and all-cause death (Virtanen et al. 2010). The association of 25(OH)D levels with all-cause and cancer mortality is conflicting (Melamed et al. 2008, Hutchinson et al. 2010, Michaëlsson et al. 2010, Freedman et al. 2010). It has been concluded that most of the evidence indicated that an improvement in vitamin D status could have a significant impact on lowering risk for peripheral vascular and cardiovascular disease (Holick 2010).

3 STUDY AIMS AND HYPOTHESES

The first aim of the present study was to determine whether relatively simple and clinically applicable physical tests could be useful in the prediction of bone density in postmenopausal women. Poor functional status has been claimed to associate with injurious falls and consequent fractures. Accordingly, the second aim was to clarify the association between physical tests and fractures.

Vitamin D deficiency is common in the elderly, and it has been associated with many health problems. The third and fourth aims were to determine if vitamin D and calcium supplementation could prevent falls and bone loss in the ambulatory general population of postmenopausal women.

4 SUBJECTS AND METHODS

4.1 Study design and subjects

The present thesis was based on Kuopio Osteoporosis Risk Factor and Prevention study (OSTPRE) and its substudy OSTPRE Fracture Prevention Study (OSTPRE-FPS). The OSTPRE study began in Kuopio, Finland, in 1989. At the baseline in 1989, the target population was 14,220 Caucasian women born in 1932-41 and living in the Kuopio province, Eastern Finland (Honkanen et al. 1991) (Figure 1).

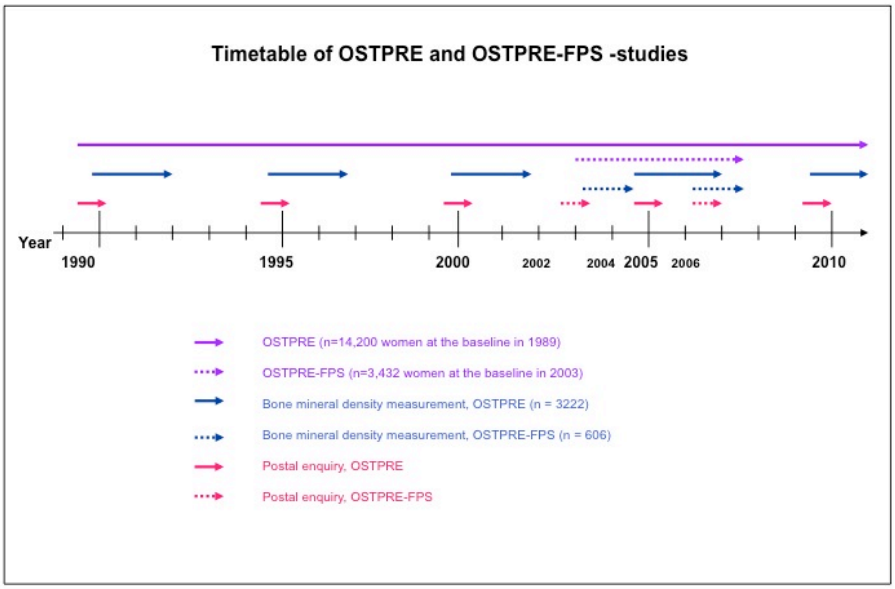

Figure 1. OSTPRE and OSTPRE-FPS studies

The OSTPRE-FPS was an open-label RCT with 3-year duration conducted during 2003-6. The primary aim of the OSTPRE-FPS was to determine whether vitamin D and calcium supplementation would be effective for fall and fracture prevention in postmenopausal women over the age of 65. The OSTPRE-FPS population (n=3,432) was randomly selected from the population-based OSTPRE cohort. The inclusion criteria for OSTPRE-FPS were the age at a minimum of 65 years at November 30th 2002, living in Kuopio province at the onset of the trial and not belonging to the OSTPRE bone density measurements sample (n=5,407). The Ethics Committee of Kuopio University Hospital approved the OSTPRE-FPS study in October 2001. The willingness to participate in a calcium and vitamin D trial was enquired via a postal enquiry from August to December 2002. The 3,432 willing women (63.5%) were randomized into two groups of equal size without blocking or stratification or random allocation sequence by an independent statistician. The subjects were informed their groups by postal notification according to the randomization. The intervention and control groups had 1,718 and 1,714 subjects, respectively. Two random samples were selected from these groups and they included 375 out of 1,718 and 375 out of 1,714 ambulatory women. These two samples formed the subsample (n=750) of this study. This subsample underwent detailed measurement program including functional capacity, serum 25(OH)D and BMD measurements (Figure 1).

The studies I, III and IV were conducted on the OSTPRE-FPS and the study II on the original OSTPRE cohort.

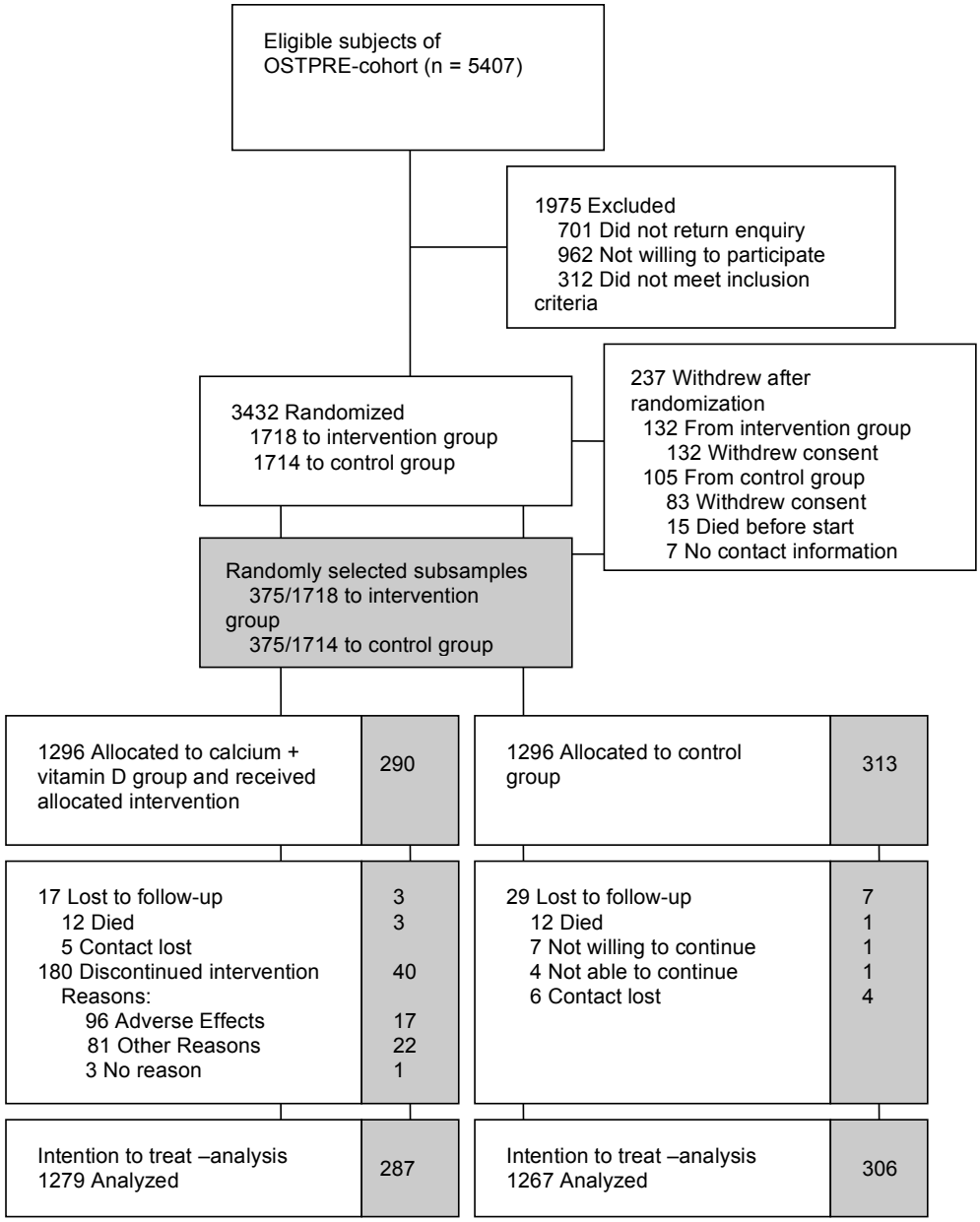

Figure 2. Flowchart of the OSTPRE-FPS trial (grey area denotes the OSTPRE-FPS subsample)

4.2 Postal enquiries

4.2.1 OSTPRE (Study II)

The postal enquiry included questions about health disorders, medication, use of hormone therapy (HT), gynecological history, nutritional habits including calcium intake, physical activity, alcohol consumption, smoking and anthropometry. At the 5-year follow-up in 1994, and subsequently at the 10-year follow-up in 1999 and the 15-year follow-up in 2004, questionnaires similar to those used at the OSTPRE baseline were sent to the 12,831, 12,562 and 12,075 women, respectively, who had responded to the first inquiry (n=13,100) and were alive at the time of enquiry. Responses were obtained from 11,954, 11,538 and 10,926 women at the five, ten and fifteen years follow-up, respectively. Thus, the response rate varied between 90-92%.

4.2.2 OSTPRE-FPS (Studies I, III and IV)

The OSTPRE-FPS baseline enquiry was sent to all who consented to participate in 2003. The enquiry information was updated from those women who had undergone bone densitometry measurements. In addition, regular exercise in the last twelve months (hours per week) was inquired with a form that listed the most popular physical activities and the duration of activity. The subjects had also a possibility to name an

activity which was not listed. The physical activity questionnaire form was designed for the OSTPRE-FPS.

4.3 Vitamin D and calcium intervention (Studies III and IV)

The intervention group received daily cholecalciferol 800 IU and calcium carbonate 1000 mg supplementation (Calcichew-D3 Forte, Leiras-Nycomed Ltd) divided into two daily doses for three years and the control group received no supplementation. The vitamin D and calcium was a combined formula in a chewable dosage form which the subjects took twice a day. Both groups were asked to continue with their previous diet. The supplements were distributed via prescriptions, which were written and supervised by the author of the study, and local pharmacies arranged the distribution of the supplements. The prescriptions were written for a one-year period at the beginning of the trial. The pharmacies had to contact the research team once a year in order to renew the prescriptions. The pharmacies provided the supplements for 3 months at a time for the subjects of the intervention group. Compliance was calculated as the percentage of the delivered tablets out of the predicted consumption during the follow-up period.

4.4 Physical tests

4.4.1 Grip strength (Studies I and II)

Grip strength (GS (kg)) of the dominant hand was measured using three successive repetitions with a Jamar hand dynamometer in OSTPRE-FPS study (Sammons Preston Rolyan, Cedarburg, WI, USA). A hand-held pneumatic squeeze dynamometer (Martin Vigorimeter, Tuttlingen, Germany) was used in the OSTPRE study. The elbow was flexed at a 90° angle and not allowed to contact any body part. Resting time between subsequent measurements was 30 seconds. The mean value of the two best performances was used in the analyses. The intraclass correlation coefficient (ICC) of the grip strength measurements has been shown to be 0.87 for absolute grip strength values (Sirola et al. 2006).

4.4.2 Standing on one foot (Studies I and II)

The subject was asked to perform a one leg stand (seconds) on the dominant foot for a maximum of 30 seconds with the eyes open. The weight-bearing foot was not allowed to move and the hands were placed on the waist. Two attempts were allowed and the longer of the two performances was recorded. The reliability coefficient for this test has previously been reported to be 0.85 (Giorgetti et al. 1998).

4.4.3 Quadriceps isometric extension strength (Studies I and II)

Quadriceps isometric extension strength (Nm) of both legs was measured by sitting in a leg extensor bench (Metitur Oy, Jyväskylä, Finland) with the femoral region in a stabilized position and the knee joint flexed at 65° at the beginning of a steady maximal isometric extension movement. For isometric quadriceps force, the coefficients for variation have been reported to be 13-19% depending on the knee movement angle (Robertson et al. 1998). Extension strength was measured three times from both legs with a 30 second rest period after each measurement. Mean values for each side were calculated using the two best performances. These were summed up to form a combined lower limb value.

4.4.4 Squatting (Studies I and II)

The subject was asked to squat down (succeeded: yes/no) vertically in a stable position and to keep her feet in place, hands straight on side, not to move, touch the floor with her fingers and then to rise up again. A moderate reliability for squatting test has been reported previously (Alaranta et al. 1994).

4.4.5 Standing with eyes closed (Study I)

Standing in the normal posture (succeeded: yes/no) was tested, with hands on the

waist, both feet on the ground and eyes closed for 10 seconds. The ICC for the test has been reported to be 0.69 (Emery et al. 2005).

4.4.6 Tandem walk and fast regular walk (Study I)

A tandem walk with hands placed on waist for six meters along a dotted line and a fast regular walk for a ten meter distance were performed. The women were asked to perform one practice before the proper measurement was done and the time of walking was measured (seconds). The ICC inter-rater reliability for the tandem walk test has been reported to be >0.88 (Rinne et al. 2001) and the test-retest reliability of the fast regular walk >0.95 (Steffen et al. 2002).

4.4.7 Chair rising test (Study I)

The number of repetitions in chair rising without the help of hands in 20 seconds was measured. The test-retest reliability (ICC) of the chair rising test has been reported to be 0.83 (Simmonds et al. 1998).

4.4.8 Vision (Study II)

The vision was measured with the standard E-board. The distance between the board and the subject was five meters. The subject had both eyes open and glasses were

allowed. She was asked to tell the first and the last mark on the line until she was unable to distinguish smaller characters. The subject was asked to enumerate the whole line, if there were errors, she was asked to read the previous line. The line where subject was able to see three letters out of five correct was recorded as the vision ability in dioptres.

4.4.9 Self-assessed ability to move (Study II)

The self-assessed ability to move (n=9,466) was obtained from the subject's self-report in OSTPRE 5-year questionnaire. This was divided into four categories, "full ability to walk and run", "ability to walk but unable to run", "walking ability maximum of one kilometer" and "walking ability maximum of one hundred meter".

4.5 Bone mineral density measurement (Studies I and IV)

BMD measurements in the OSTPRE-FPS were performed at the lumbar spine (L2-L4), left proximal femur and total body with DXA (Lunar Prodigy, GE, Madison, WI, USA). The regions of interest at the proximal femur included femoral neck, trochanter, Ward's triangle and total proximal femur. The quality control of the DXA instruments was run daily. The in vitro long-term reproducibility (CV) for total femur region was 0.3%. Technical quality of each DXA measurement and analysis was carefully checked and

those with measurement errors were not included in the statistical analyses.

4.6 Falls (Study III)

The falls were defined according to the ICD10 disease classification including the categories W00-W03 (fall on same level), W10 (fall on stairs) and R29.6 (tendency to fall). The participants in the subsample were telephoned initially four months after the baseline measurement and then at four month intervals to record the incidence and circumstances of falls, any resulting injuries and whether the fall was so serious that it required medical attention. The rest of the trial population was interviewed by phone once a year between January and April during the trial.

4.7 Fractures (Study II)

The follow-up time for fractures started at the five-year measurement of the OSTPRE study (1994-1997) and lasted until the 1st of October 2004 if no fracture was recorded. The total duration of follow-up varied from 6.4 to 9.9 (mean 8.4) years. Fractures due to high-energy trauma, e.g. motor vehicle and bicycle crash, falls from above the ground level and pathological process were excluded. Follow-up fractures were recorded based on ten-year and fifteen-year follow-up questionnaires. The women were questioned about whether they had suffered a fracture during the preceding 5-year period and its type, mechanism and treatment. All self-reported fractures were validated by cross-

checking with radiological reports. However, in cases where a rib fracture was reported, the diagnosis was also accepted based on clinical assessment without radiological proof. The self-reported fractures in the OSTPRE-study have been previously validated (Honkanen et al. 1999). Fracture history was recorded from the OSTPRE baseline to the 5-year measurement.

4.8 Statistical analyses

All statistical analyses were carried out using the Statistical Package for Social Sciences (version 14.0.1; SPSS, Chicago, IL) for Windows. In the cross-sectional study (I), analyses were performed with multivariate linear regression and two-way ANOVA models. Receiver operator characteristics (ROC) curves and the corresponding area under the curve (AUC) were used to evaluate how well physical tests could predict osteoporosis. The analyses were adjusted for age (years), body mass index (BMI) (cm/m^2), years of HT, years since menopause, a dichotomous current smoking (yes/no) and the use of oral glucocorticoids.

In the cohort study (II), Cox regression analyses were used to analyze the ability of physical tests to predict future fractures during 8-year follow-up. The results were expressed as hazard ratios (HR) with 95% confidence intervals (95% CI). The analyses were adjusted for age, BMI, years of HT, years since menopause, current smoking status (yes/no) and fracture history (yes/no).

In the RCT study (III) data was analysed on intention-to-treat (ITT) basis by retaining allocation to groups according to randomization and by including all subjects with endpoint information. The primary outcomes were the women with falls and women with falls requiring medical attention. The posthoc analyses included multiple falls and multiple falls requiring medical attention. The proportion of individuals falling in intervention vs. control group was analysed with the risk ratios (RR). The risk (OR) of falling was analysed with logistic regression, in which the dependent variable was either a person with 0 vs. one with 1+ falls or a person with 0-1 falls vs. one with 2+ falls.

In the RCT study (IV) data was analysed on the ITT basis by retaining allocation to groups according to randomization. The change in BMD inside the group was defined with paired samples t-test. The change of BMD between the groups was defined with general linear repeated measures model (GLM). All analyses were un-adjusted.

The change in physical capacity between the RCT intervention and control groups was analysed with the GLM. The GLM analyses included the baseline and the end-point measurements of the physical capacity tests. The association between the physical capacity tests and the falls was assessed with logistic regression.

5 RESULTS

Table 1. The main results from the studies I-IV

Study	Hypothesis	Study population	Main results
I	Physical tests associates with osteoporosis	n=606, cross-sectional study, OSTPRE-FPS	Grip strength, standing on one foot and ability to squat down to floor associated with BMD
II	Physical tests predict fractures	n=2928/9466, prospective cohort study with follow-up of 8.4 years, OSTPRE	Standing on one foot < 10 seconds, grip strength and ability to walk < 100 meters predict postmenopausal fractures
III	Vitamin D and calcium decrease the risk of falls	n=3139, RCT with follow-up of 3 years, OSTPRE-FPS	Vitamin D 800IU and calcium 1000mg decreased multiple falls requiring medical attention (n=3139) by 28% and multiple falls (n=593) by 30%
IV	Vitamin D and calcium have positive skeletal effect	n=593, RCT with follow-up of 3 years, OSTPRE-FPS	Vitamin D 800IU + calcium 1000mg prevents postmenopausal bone loss

5.1 Characteristics of the study population

Table 2. Baseline characteristics of the 2928 OSTPRE women in 1994 (Study II)

Characteristics	
Age (SD)	59.1 (2.9)
Weight (kg) (SD)	72.1 (13.2)
Height (cm) (SD)	160.0 (5.7)
Years postmenopausal (SD)	6.7 (5.0)
Use of hormone therapy (years) (SD)	2.6 (3.5)
Smoker at baseline, n (%)	269 (9.1)
Previous fracture[a], n (%)	363 (12.3)
Grip strength (Nm) (SD) (n=2904)	68.0 (18.0)
Isometric knee extension strength (kg) (SD) (n=1767)	31.6 (11.6)
Vision (dioptre) (SD) (n=1822)	1.2 (0.3)
Ability to squat down (%) (n=2885)	96.0
Ability to squat down to the floor (%) (n=2809)	74.7
Standing-on-one-foot at least 10 seconds (%)(n=2844)	93.0

[a] *wrist 103/3.5%, ankle 58/2.0%, clinical vertebral 20/0.7%, hip 6/0.2% and all other fractures 176/6.0%*

Table 3. Baseline characteristics of the subsample (n=593) and the entire study population (n=3139) of the OSTPRE-FPS in 2004 (Studies I, III and IV).

	Subsample (n=593)		Entire study population (n=3139)	
Characteristic	**Intervention group (n=306)**	**Control group (n=287)**	**Intervention group (n=1566)**	**Control group (n=1573)**
Age (years)	67.4 (2.0)	67.4 (1.9)	67.4 (1.9)	67.3 (1.8)
Weight (kg)	70.3 (12.3)	69.9 (10.3)	70.7 (12.4)	70.8(11.5)
Height (cm)	160 (5.3)	160 (5.1)	160 (5.3)	160 (5.3)
BMI (kg/m^2)	27.5 (4.5)	27.4 (3.9)	27.7 (4.6)	27.7 (4.4)
Age at menopause (years)	49.3 (5.3)	49.4 (4.6)	49.3 (4.7)	49.6 (4.5)
Years since menopause	18.1 (5.7)	18.1 (5.2)	18.2 (5.0)	17.8 (5.0)
History of HT-use (%)				
Users	52.9 %	56.5 %	52.3 %	52.4 %
Duration of HT-use (years)	10.6 (6.6)	11.4 (7.6)	10.5 (6.9)	10.3 (7.4)
Use of calcium supplements	15.0 %	19.0 %	16.2 %	18.0 %
Calcium/milk products (mg/day)	928 (487)	888 (490)	892 (472)	892 (472)
Calcium/supplements (mg/day)	310 (179)	319 (180)	321 (191)	312 (187)
Calcium total (mg/day)	988 (490)	965 (489)	952 (485)	961 (482)
Number of prescribed medications	2.8 (2.6)	2.5 (2.5)	2.7 (2.6)	2.6 (2.6)
Alcohol doses/week	0.7 (1.5)	0.8 (1.4)	0.8 (1.5)	0.8 (1.5)
Alcohol doses/week (%)				
no alcohol	52.3 %	45.6 %	48.7 %	49.9 %
<1 doses / week	22.0 %	24.9 %	23.6 %	22.9 %
1 – 4.9 doses / week	23.8 %	26.2 %	25.0 %	24.4 %
5 – 9.9 doses / week	1.4 %	3.4 %	2.3 %	2.3 %
>= 10 doses /week	0.5 %	0.0 %	0.5 %	0.4 %
Smoking status (%)				
Current smoker	6.2 %	3.6 %	4.8 %	5.5 %
Physical activity level (hours)	11.0 (10.8)	12.4 (12.8)	-	-
Self-assessed physical ability [1]				
Capable of running	41.5 %	55.7 %	48.0 %	51.1 %
Capable of walking	49.6 %	36.3 %	42.8 %	39.5 %
Walking max 1000 m	6.0 %	6.0 %	6.4 %	7.3 %
Walking max 100 m	1.8 %	1.3 %	1.8 %	1.5 %
Walking indoors	1.1 %	0.7 %	0.9 %	0.5 %
Unable to walk	0.0 %	0.0 %	0.1 %	0.1 %
Bone mineral density				
Femoral neck (g/cm2)	0.866 (0.132)	0.866 (0.120)	-	-
L2-L4 (g/cm2)	1.082 (0.189)	1.096 (0.190)	-	-
Serum 25(OH) level	50.0 (18.7)	49.1 (17.7)	-	-

[1] *The difference was statistically significant at the p=0.01 level in the subsample.*

5.2 Physical tests and osteoporosis (Study I)

Table 4. Association (r^2) between physical tests and osteoporosis

Physical test	Lumbar spine	Femoral neck	Trochanter	Total proximal femur
Standing on one foot (s)	0.16[1]	0.17[2]	0.20[2]	0.23[2]
Grip strength (Nm)	0.16[1]	0.16[1]	0.19[2]	0.21[1]
Squat down	-	0.15[1]	0.19[1]	0.21[1]

[1] *The association was statistically significant at the p<0.05 level*
[2] *The association was statistically significant at the p<0.001 level*

Standing on one foot (SOOF) was associated with lumbar spine (r^2=0.16, p=0.004), femoral neck (r^2=0.17, p < 0.001), trochanter (r^2=0.20, p < 0.001) and total proximal femur BMD (r^2=0.23, p < 0.001). The grip strength (GS) was associated with lumbar spine (r^2=0.16, p=0.011), femoral neck (r^2=0.16, p=0.002), trochanter (r^2=0.19, p < 0.001) and total proximal femur BMD (r^2=0.21, p=0.004). The ability squat down to floor was associated with the femoral neck (r^2=0.15, p=0.031), trochanter (r^2=0.19, p=0.040) and total proximal femur BMD (r^2=0.21, p=0.028).

According to the ROC analyses, identifying women at risk of osteoporosis using a threshold of 22kg in GS would yield a true-positive rate (sensitivity) of about 58%, but there would be a false positive rate (1-specificity) of 14%. This means that a total of 58% of the women with osteoporosis would be included into this group, and only 14% who were predicted to have osteoporosis would not be suffering from this disease. A threshold of 16 seconds in the SOOF test would yield a true-positive rate of about 67% and a false positive rate of 40%. A less stringent threshold would identify more women with osteoporosis, but would increase the rate

of overdiagnosis.

The subjects were divided into normal/osteopenic and osteoporotic groups according to the T-score of the femoral neck and lumbar spine BMD (WHO classification). Osteoporotic women, according to femoral neck, performed significantly worse in SOOF (p=0.001), GS (p<0.001), leg extension strength (p=0.007), ability to squat down to floor (p=0.004) and tandem walk for 6 meters (p=0.014). According to the lumbar spine BMD, SOOF (p=0.032), GS (p=0.005) and leg extension strength (p=0.037) were significantly worse in osteoporotic subjects.

5.3 Physical tests and fractures (Study II)

Table 5. Associations (HR with 95% CI) between physical tests and fractures

Physical test	Hip fracture	Clinical vertebral	All fractures
Inability to stand on one foot for 10 seconds	9.11 (1.98-42.00) [2]	-	-
Grip strength (per 1Nm decrease)	1.05 (1.01-1.09) [1]	-	-
Knee extension strength (per 1kg decrease)	-	-	1.02 (1.00-1.03) [1]
Ability to walk less than 100 meters	11.57 (2.73-49.15) [2]	3.85 (1.45-10.22) [2]	-

[1] *The association was statistically significant at the p<0.05 level*
[2] *The association was statistically significant at the p<0.01 level*

The inability to SOOF for 10 seconds increased the risk of subsequent hip fracture (HR with 95% CI, significance) 9.11-fold (1.98-42.00, p=0.005). Decreased grip strength associated with 1.05-fold (1.01-1.09, p=0.026) increased risk of hip fractures. Low leg extension strength associated with 1.02-fold (1.00-1.03, p=0.013) higher risk for all fractures. The HRs are

estimated per one Newton meter and one kg decrease in grip and leg extension strength, respectively. No significant relationships were found between physical tests with wrist and ankle fractures. The self-assessed ability to walk less than 100 meters increased the risk of ankle fractures by 2.36-fold (1.10-5.08, p<0.05), hip 11.57-fold (2.73-49.15, p<0.01) and clinical vertebral fractures by 3.85-fold (1.45-10.22, p<0.01).

5.4 Vitamin D and calcium supplementation and falls (Study III)

Table 6. The effect (OR with 95% CI) of daily vitamin D 800IU and calcium 1000mg supplementation on falls and falls requiring medical attention

Endpoint	**Entire trial population (n=3139)**	**Subsample (n=593)**
Falls	1.05 (0.91-1.20)	0.82 (0.58-1.14)
Multiple falls	1.13 (0.97-1.32)	0.70 (0.50-0.97) [1]
Falls requiring medical attention	0.84 (0.70-1.01)	0.93 (0.66-1.31)
Multiple falls requiring medical attention	0.72 (0.53-0.97) [1]	0.82 (0.49-1.37)

[1] *The odds ratio was statistically significant at the p<0.05 level*

In the entire trial population (n=3139), a total of 1645 subjects with 3776 falls were recorded. There were 812 (out of 1566, 52%) women experiencing 1832 falls in the intervention group and 833 (out of 1573, 53%) women with 1944 falls in the control group (risk ratio (RR) for the number of fallers was 0.98, 95% CI 0.92-1.05, p=0.160). In the logistic regression model (subject with none or one fall versus subject with two or more falls), the results were also statistically non-significant (odds ratio (OR) 1.13, 95% CI 0.97-1.32, p=0.113). The comparison of those individuals with no falls versus individuals with a fall was statistically non-significant

(OR 1.05, 95% CI 0.91-1.20, p=0.536).

A total of 384 subjects experiencing 954 falls were recorded during the three year follow up in the subsample (n=593). There were 179 (out of 287, 62%) women with 430 falls in the vitamin D and calcium supplementation group and 205 (out of 306, 67%) women with 524 falls in the control group (RR 0.82, 95% CI 0.73-0.92, p=0.032). In the logistic regression model (an individual with none or one fall versus an individual with two or more falls), the multiple fall incidence decreased by 30% in the supplementation group (OR 0.70, 95% CI 0.50-0.97, p=0.034). In the comparison of those subjects with no falls versus subjects with at least one fall, the results were statistically non-significant (OR 0.82, 95% CI 0.58-1.14, p=0.239).

In the entire trial population (n=3139), a total of 557 subjects with 821 falls requiring medical attention (FRMA) were recorded. There were 258 (out of 1566, 16%) women with 377 FRMA in the intervention group and 299 (out of 1573, 19%) women with 444 FRMA in the control group. In the logistic regression model (a subject with none or one FRMA versus a subject with two or more FRMA), the FRMA incidence decreased by 28% in the supplementation group (OR 0.72, 95% CI 0.53-0.97, p=0.031). Comparing individuals with no FRMA versus individuals with FRMA, the results were statistically non-significant (OR 0.84, 95% CI 0.70-1.01, p=0.063).

A total of 201 subjects with 301 FRMA were recorded during the three-year follow up in the subsample (n=593). There were 95 (out of 287, 33%) women with 142 FRMA in the vitamin D and calcium supplementation group and 106 (out of 306, 35%) women with 159 FRMA in the control group. In the logistic regression model (a subject with none or one FRMA versus a

subject with two or more FRMA), the results were statistically non-significant (OR 0.82, 95% CI 0.49-1.37, p=0.443). The comparison of those subjects with no FRMA versus subjects with FRMA was statistically non-significant (OR 0.93, 95% CI 0.66-1.31, p=0.692).

5.5 Vitamin D and calcium supplementation and bone loss (Study IV)

Table 7. The effect (percentage change) of daily vitamin D 800IU and calcium 1000mg supplementation with adherent women on bone loss

Study group	Spine (L2-L4) [2]	Femoral neck[2]	Trochanter[2]	Proximal femur[2]	Total body[1]
Intervention (%)	0.67	-1.26	0.25	-0.84	1.31
Control (%)	0.76	-1.73	-0.88	-1.47	0.19

[1] *The difference was statistically significant at the p<0.05 level*
[2] *The difference was statistically significant at the p<0.01 level*

Total body BMD increased significantly more in the intervention group than in the control group (0.84% vs. 0.19%, p=0.011). The BMD decrease at the Ward's triangle was lower in the intervention group (-2.69% vs. -2.83%, p=0.003). The BMD changes at the spine (p=0.372), femoral neck (p=0.188), trochanter (p=0.085) and total proximal femur (p=0.070) were statistically non-significant between the groups.

The women who were adherent (i.e. those who took at least 80% of their supplementation) showed significantly lower bone loss in femoral neck (-1.26% vs. -1.73%, p=0.002), Ward's triangle (-1.63% vs. -2.83%, p<0.0001), trochanter (0.25% vs. -0.88%, p=0.001) and total proximal femur (-0.84% vs. -1.47%, p<0.0001) than the control group. Further, total body BMD increased more in the intervention group (+1.31% vs. +0.19%, p=0.002). In contrast, lumbar

spine bone loss was greater in the intervention group than in the control group (+0.67% vs. +0.76%, p=0.033).

5.6 Vitamin D and calcium supplementation and physical capacity

There were no statistically significant changes in the physical capacity tests between the study groups. Furthermore, the physical capacity did not change differently in subjects with a single or multiple falls (Table 8).

Table 8. Physical capacity during three year trial in intervention (n=306) and control (n=287) groups in the subsample of the OSTPRE-FPS and difference of change between the groups (p-value; GLM-test)

Physical capacity test	**Baseline, mean (SD)**	**3-years, mean (SD)**	**p-value**
Grip strength, kg			0.211
Intervention group	26.0 (5.1)	25.0 (5.9)	
Control group	26.6 (7.4)	25.0 (5.3)	
Standing on one foot, seconds			0.059
Intervention group	19.2 (10.5)	16.7 (11.3)	
Control group	18.5 (10.5)	17.6 (10.9)	
Ability to squat down to floor, yes/no			0.477
Intervention group	0.7 (0.5)	0.7 (0.5)	
Control group	0.7 (0.5)	0.7 (0.5)	
Standing with eyes closed 10 seconds, yes/no			0.861
Intervention group	0.9 (0.2)	1.0 (0.2)	
Control group	1.0 (0.2)	1.0 (0.2)	
Chair rising test, number of repetitions			0.279
Intervention group	8.0 (4.4)	8.4 (3.2)	
Control group	7.9 (2.7)	8.7 (3.1)	
Tandem walk 6m, seconds			0.971
Intervention group	18.7 (7.7)	18.5 (13.7)	
Control group	19.7 (8.7)	19.4 (10.9)	
Regular walking 10m, seconds			0.236
Intervention group	6.4 (1.9)	6.5 (3.0)	
Control group	6.1 (1.5)	6.8 (2.5)	
Right leg extension strength, Nm			0.828
Intervention group	304.2 (79.3)	275.8 (73.1)	
Control group	297.0 (78.6)	267.3 (69.6)	
Left leg extension strength, Nm			0.324
Intervention group	287.3 (77.7)	265.4 (69.1)	
Control group	290.9 (85.3)	262.7 (70.0)	

5.7 Physical capacity tests and falls

Standing-on-one-foot time was associated with a decreased risk for falls (individual with no falls versus individual experiencing a fall) (OR for one sec. increase 0.98, 95% CI 0.97-0.99, p=0.026). Further, the standing-on-one-foot test was associated with multiple falls (individual with no falls or one fall versus individual with two or more falls) (OR for one sec. increase 0.98, 95% CI 0.96-0.99, p=0.005).

5.8 Compliance and adverse effects of the supplementation (Studies III and IV)

The mean compliance for vitamin D and calcium supplementation was 78%. The numbers for 70%, 80% and 90% compliance were 77.4%, 74.2% and 69.1% of the intervention group (entire trial population), respectively.

A total of 220 out of 1586 women discontinued the intervention, 113 due to adverse effects, 103 due to other reasons and four did not give any specific reason. The most common adverse effects causing discontinuation were gastrointestinal symptoms (64 cases out of the entire intervention population, 1586), nausea (12 out of 1586) and skin reactions (9 out of 1586). Gastrointestinal symptoms included abdominal pain and heartburn. Other reasons for discontinuation the study included: not willing to continue intervention, pharmacy stopped intervention delivery, fear of interactions, onset of illness, not willing or capable to visit pharmacy, primary health care physicians decision, bad taste of tablets, tablets difficult to

swallow, fear of accumulation of calcium, considered that she was receiving sufficient calcium and vitamin D from other sources, fear of orange flavour, considered tablets as placebo and participation in another trial.

6 DISCUSSION

6.1 Methodological considerations

6.1.1 Study population

The target population at baseline in 1989 was all of the 14,220 Caucasian women born in 1932-41 and living in the Kuopio province in Eastern Finland. The response rates to the OSTPRE postal enquiries were high (90-92%). Thus the OSTPRE cohort represents fairly well the target population.

The study population in studies I, III and IV originated from the OSTPRE-FPS. The cohort in study II was from the OSTPRE. The 5,407 subjects were selected from the original OSTPRE cohort to participate in the OSTPRE-FPS and the 3,432 volunteers (63.5%) were included. Consequently, the population-based benefit was diminished. With this limitation, the generalization of the results of the studies III and IV to the Caucasian female population over the age of 65 years might still be justified.

Participation in the trial might have resulted in over-representation of healthier women in OSTPRE-FPS study. This was also confirmed, since in the loss analysis, the subjects, who left the study between the randomization and beginning of the trial, were heavier, with higher BMI, used less HT and alcohol but more cigarettes, and had poorer self-assessed physical capacity.

In all, 3.8% of control subjects reported using prescribed vitamin D with or without calcium supplements at the baseline, but this has risen to 16.1% by the end of the trial. This, in combination with a mean compliance in the calcium plus vitamin D group of 78%, may have decreased the difference in calcium and vitamin D intake between the groups, and weakened the power to detect a difference. Adherence rates of less than 60% have been suggested to be insufficient to achieve a fracture prevention effect (Bischoff-Ferrari et al. 2007). In addition, large trials with vitamin D for the prevention of fractures among older persons indicate that only about 50% of older individuals adhere to a daily oral vitamin D supplement (Grant et al. 2005, Porthouse et al. 2005). Accordingly, the compliance of 78% of the present study might be considered as acceptable and high enough to reveal any positive effect of vitamin D and calcium supplementation. To sum up, younger age, healthier study population, incomplete compliance and increased supplementation use in the control group as well as relatively high nutritional calcium intake and serum 25(OH)D levels may have jointly decreased the size of the effect in this study.

6.1.2 Study design

Study I was cross-sectional which means that no conclusions can be drawn about causal relationships. Its purpose was to find diagnostic tools for osteoporosis. Study II was an observational epidemiological study which may have uncontrolled confounding. However, population-based study sample may diminish confounding.

Studies III and IV were randomized controlled trials (RCT). The RCT studies are reputed to

represent the highest grade in the hierarchy of research designs (Benson et al. 2000, Concato et al. 2000). However, OSTPRE-FPS was an open trial and neither placebo control nor blinding was applied. The reason for blinded assessment and placebo is to prevent biased reporting. It could be that subjects in the intervention group of study III recalled falls more completely since they were more intensively involved in the trial. This could have lead to differential misclassification resulting in underestimation of the effect. However, the average time of phone contacts was similar in both groups, which should have decreased the differential recall. The results were also consistent between the falls in general and falls requiring a medical attention which support the validity of the findings.

The OSTPRE-FPS a priori power calculations were conducted based on fracture incidence from an earlier Swedish study (Kanis et al. 2001). Accordingly, the primary endpoint of the OSTPRE-FPS study was a fracture. However, the statistically significant results in studies III and IV reveal to some extent that there was a sufficient sample size also for the secondary endpoints.

The follow-up time of three years in OSTPRE-FPS was long enough to detect both fall and BMD endpoints. However, with respect to fractures, a follow-up period of five years could have been better since the fracture incidence remained rather low in the present study.

6.1.3 Measurements and collection of outcome events

All physical capacity and muscle strength tests in the study I were first carefully explained and

demonstrated to the subjects and the performance was controlled by specially trained personnel. Grip strength measurement was the only physical test that was validated in our cohort (Sirola et al. 2006). However, the validity of physical tests has been shown to range from good to moderate in earlier studies (Alaranta et al. 1994, Giorgetti et al. 1998, Robertson et al. 1998, Simmonds et al. 1998, Rinne et al. 2001, Steffen et al. 2002, Emery et al. 2005, Sirola et al. 2006).

The BMD measurements in the studies I and IV were performed at the lumbar spine (L2-L4), left proximal femur and total body with DXA (Lunar Prodigy, GE, Madison, WI, USA). Errors due to measurement technique were minimized by using specially trained personnel and by regular use of phantom measurement controls.

Follow-up fractures in study II were recorded based on OSTPRE ten-year and fifteen-year follow-up questionnaires. The women were questioned about whether they had suffered a fracture during the preceding 5-year period and its type, mechanism and treatment. All self-reported fractures were validated by cross-checking with radiological reports. The validity of self-reports of fractures in OSTPRE study in general has been previously assessed (Honkanen et al. 1999).

The inadequacy of infrequent (annual) fall assessment in study III is illustrated by the higher fall rate documented in the subset of 593 subjects in whom falls were assessed every 4 months compared with the other subjects in whom falls were assessed annually. Even in the subsample, a phone call every 4 months without a diary or a postcard note on each fall may have limitations. It has been reported that the amount of time elapsing between the exposure

and the recall is an important indicator of the accuracy of recall (Rothman et al. 1998). However, previously used reporting methods (Graafmans et al. 1996, Bischoff-Ferrari et al. 2003) might not have had as good adherence in a population-based study. Since fall reporting is based on self-assessment, it is virtually impossible to achieve the same kind of accuracy as with fracture reporting. The problem with fall reporting is that most falls do not cause any injury, and thus they are soon forgotten. It has been recognized that recall of falls has only limited accuracy (Cummings et al. 1988, Kanten et al. 1993). The placebo controlling might have increased the validity of self-reported falls. Use of medical records for fall validation might not confer any additional validity since the reason for hospital visits might not have been fully recorded. The falls requiring medical attention were also used since one could argue that this outcome will have been more completely reported than falls in general. The observed odds ratios were probably closer to the null than would have been the case if the recall bias had been absent. Although there are limitations in fall reporting, it is believed that the pragmatic approach of community-based intervention has advantages compared with earlier studies which have limited generalization to the overall women population over the age of 65 years.

6.2 Physical tests and osteoporosis

In study I, BMD associated with SOOF, GS and ability squat down to floor. It has been shown that it is worthwhile to recommend BMD testing to women aged 65 or older or younger postmenopausal women who have a strong or multiple risk factors for suffering fracture (US Preventive Services Task Force 2002). However, most individuals with osteoporosis remain undiagnosed and untreated (Kiebzak et al. 2002, Gehlbach et al. 2002, Morris et al. 2004).

Several clinical risk factors for low bone mass have been found; these include female sex, increased age, estrogen deficiency, white race, low weight and BMI, family history of osteoporosis, smoking and history of prior fracture (National Institutes of Health 2001, Rosen 2005, Kanis et al. 2008). Different risk factors have been combined to form risk assessment tools and these have been further evaluated (Cadarette et al. 2001, Gourlay et al. 2008). However, none of these have included physical performance as a clinical risk factor for osteoporosis.

BMD is an intermediate endpoint. Furthermore, BMD measurements cannot identify those subjects who will suffer a fracture (Marshall et al. 1996). Fracture outcome could be considered more clinically important. Physical capacity tests reflect indirectly on neuromuscular function as muscle force, muscle power and balance, are all contributive factors for falls and fractures. Bone medications are used throughout the world to prevent fractures in patients with osteoporosis (Liberman et al. 1995, Saag et al. 1998, Recker et al. 2004). Accordingly, associations between physical capacity tests and osteoporosis might help clinicians to objectively determine which patients should be further evaluated for osteoporosis and fracture prevention.

There were 22 (4%) and 61 (12%) subjects suffering from osteoporosis of the femoral neck and lumbar spine, respectively. In a Swedish population sample, the prevalence of femoral neck osteoporosis in the same age group has been 20% (Kanis et al. 2000). Accordingly, the low number of osteoporotic subjects in this present study might indicate that this was a healthier population cohort. Further, the low absolute number of osteoporotic subjects might have caused some of the analyses to remain underpowered to be able to detect statistically

significant results.

Unexpectedly, BMD of the lower limb was not associated with leg extension strength or chair rising test. Previously, neuromuscular performance has been shown to independently associate with bone strength (Rantalainen et al. 2010). However, femoral BMD was associated with SOOF and ability in the squat down to floor tests which measures not only muscle strength but also balance and coordinative muscle function. Based on this finding, it could be postulated that coordinative muscle function and muscle power of the lower limbs might be associated with BMD rather than muscle strength alone. In addition, the changes in muscle strength with age have been shown to differ between different muscle groups (Rantanen et al. 1997).

Previously, it has been shown that spinal motion performance declined and functional impairment increased in relation to the severity of bone loss (Tsauo et al. 2002). Osteoporosis has been associated with back and functional impairment, which further affected the quality of life (Tsauo et al. 2002, Fechtenbaum et al. 2005). The questionnaire about the back pain, physical test which measures lower back mobility and their association with osteoporosis will need to be assessed in more detail in future studies.

BMD measurements can be used to estimate quantity of bone. In addition to quantity, bone quality is an important determinant of the fracture risk (Stokstad 2005, Seeman et al. 2006). In the future studies, one further focus might be to clarify possible associations between bone geometry, structure and muscle performance.

6.3 Physical tests and fractures

In study II, the decreased GS, low leg extension strength, inability to SOOF 10 seconds and self-assessed ability to walk less than 100 meters were associated with future fractures. It has been shown that half (Sanders et al. 2006) or even more than half (Pasco et al. 2006) of fragility fractures in women over the age of 50 occur in women without osteoporosis. Even if one considers subjects suffering the classical "osteoporotic" fractures (hip, Colles and vertebral) then only 59% have osteoporosis (Sanders et al. 2006). Accordingly, other clinical risk factors in addition to BMD provide supplemental insight into the prevention of fractures (Taylor et al. 2004, Kanis et al. 2007). However at present, clinical risk factors do not include physical capacity measurement (Kanis et al. 2008) although grip strength has been shown to predict fractures in perimenopausal women (Sirola et al. 2006, Sirola et al. 2008). The WHO fracture risk assessment tool FRAX^{TM} is based on individual patient models that integrate the risks associated with clinical risk factors but it does not include the physical capacity. The important risk factors for hip and wrist fractures apparently are related to bone strength and falls (Nevitt et al. 1993, McClung et al. 2001). Thus, the associations between physical capacity and fractures could provide new methods for the fracture prevention in the future.

In women who have experienced a fall, limitations in mobility have been shown to increase the risk for future falls by 4 to 15-fold (Mänty et al. 2010). Falls are an important external cause of fractures to distal radius (Vogt et al. 2002, Honkanen 1995), proximal humerus (Kristiansen et al. 1987) and hip (Hayes et al. 1993). Accordingly, the risk for fracture-related falls has been studied. Slow gait speed, difficulties in tandem walk and poor vision (Dargent-Molina et al.

1996, Taylor et al. 2004), impaired physical function (Formiga et al. 2008), balance (Wagner et al. 2009) and self-reported health and physical activity (Robbins et al. 2007) have been associated with hip fractures. Low lower limb strength may increase the relative risk for by falls ten fold (Carter et al. 2001). However, subjects in study II were rather young since their mean baseline age was 59. In this population, wrist and ankle fractures were the most common. Falls and BMD have been associated with wrist fractures (Vogt et al. 2002, Honkanen et al. 1995) and lifestyle factors (i.e. smoking and obesity) with ankle fractures (Valtola et al. 2002). It might be that people who are more active sustain more often falls and subsequently fractures. On the other hand, it might be that the nature of the fall is an important predictor of these fractures. For example, subjects with poor physical capacity might have poorer protective responses to falling. In fact, a fall to the side and impairment in mobility are important risk factors for hip factures in osteoporotic elderly individuals (Greenspan et al. 1998). In addition, obese subjects might have a higher fall impact owing to body weight.

Frailty in older adults as defined by unintentional weight loss (10 lbs in past year), self-reported exhaustion, muscle weakness (grip strength), slow walking speed, and low physical activity, have all been shown to increase with age (Fried et al. 2001). In women over the age of 60, the risk of fracture has been shown increase by 6% per year (Pasco et al. 2006). Accordingly, frailty and its increasing prevalence during aging obviously is important in osteoporosis as well as in fall and fracture risk.

Hand grip strength has been shown to be an indicator of overall muscle strength and it may predict mortality via mechanisms other than those leading from disease to muscle impairment (Rantanen et al. 2003). Furthermore, grip strength and SOOF were associated with lumbar

spine BMD in study I but these tests were not associated with clinical vertebral fractures in study II. Although femoral BMD is more strongly related to most of the fracture types than spinal BMD in the elderly (Stone et al. 2003) spinal BMD has been shown to be independently related with vertebral fractures (Cauley et al. 2007). Bending and lifting activities have been shown to generate loads on the spine that in very low BMD can exceed the failure load (Myers et al. 1997). That may be that reason why some part of vertebral fractures might have been not due to a fall and therefore increasing muscle strength in an attempt to avoid the falls has not been meaningful. In fact, spinal BMD might be a better indicator for fall related fracture risk in younger people (Honkanen et al. 1995).

In the study population of early postmenopausal women, it is likely that there are some comorbid neurological or muscular conditions or other issues that may increase fracture risk for those who cannot stand on one foot for 10 seconds or who have hip fractures. However, comprehensive adjustment for known confounders (age, BMI, years of HT, years since menopause, current smoking status and fracture history) was used to minimize bias caused by those factors. One limitation was that there were only eight hip fractures in this relatively young group of women.

A history of prior fracture is a strong risk factor for future fractures, an over and above that can be explained by variations in BMD (Kanis et al. 2004). Mortality after low-trauma fracture has been associated with quadriceps weakness and subsequent fracture (Bliuc et al. 2009). In addition, the disability following fractures has been studied (Fink et al. 2003). Accordingly, there might possibly be causality between physical capacity, fractures and mortality.

6.4 Vitamin D and calcium supplementation and falls

It was found that vitamin D and calcium supplementation was effective in preventing multiple falls. This finding is supported by Bischoff et al. who have demonstrated previously that recurrent fallers benefit most from this kind of treatment (Bischoff et al. 2003). As discussed above, study III had the possible limitation on some degree of non-accurate self-reporting of falls. However, this study is the first to demonstrate the effective role of vitamin D and calcium supplementation on injurious fall in a general population women aged 65 to 71.

Previous studies about the effect of vitamin D and calcium supplementation on falls have been conducted in the very elderly or institutionalised subjects (Bischoff et al. 2003, Bischoff-Ferrari et al. 2004, Pfeifer et al. 2009). However, even in the very elderly, some studies have been negative (Burleigh et al. 2007). The low prevalence of very low 25(OH)D levels have been used as an explanation for the lack of association between falls and vitamin D levels (Pramyothin et al. 2009). Another study detected the most pronounced effect in less physically active women (Bischoff-Ferrari et al. 2006).

In future studies, the effect of a higher vitamin D dose might reveal additional benefit for fall prevention. It might be that the present dose was not sufficient to reveal all potential to muscle performance. One could argue that especially vitamin D was the key nutrient since the mean nutritional calcium in present population intake was sufficient. According to the recent American Society for Bone and Mineral research statement the beneficial effects of calcium occur at relatively low doses (American Society for Bone and Mineral Research 2010). Thus,

the need for additional calcium supplementation in addition to the nutritional supply of 1000mg should be examined critically.

6.5 Vitamin D and calcium supplementation and bone loss

It was found that bone loss in ambulatory women could be decreased by vitamin D and calcium supplementation. There is consistent evidence that vitamin D deficiency can evoke accelerated bone loss in elderly subjects (Krall et al. 1989, Dawson-Hughes et al. 1991). The effect of vitamin D on improved bone strength and mass has been attributed to lower PTH levels and consequently lower stimulus for bone resorption (Mezquita-Raya et al. 2001, Dawson-Hughes et al. 2007). A recent study indicated that only women with 25(OH)D levels below 50nmol/l benefits from a higher calcium intake (Bischoff-Ferrari et al. 2009). A meta-analysis revealed that calcium alone and calcium with vitamin D were associated with reduced bone loss at the hip and spine (Tang et al. 2007).

Femoral neck BMD has been reported to fluctuate with the seasons (Dawson-Hughes et al. 1991). Dawson-Hughes et al. reported that 500mg of calcium and 700IU of vitamin D_3 supplementation had no effect on proximal femur in a three-year follow-up (Dawson-Hughes et al. 1997). However, an increase of femoral BMD during a shorter 18 months follow-up has also been reported with 1200mg of calcium and 800IU of vitamin D_3 (Chapuy et al. 1992). Chapuy et al. described a lower rate of annual bone loss at the proximal femur in 1200mg of calcium and 800IU of vitamin D_3 supplemented subjects (Chapuy et al. 2002) but 1000IU vitamin D_2 treatment had no effects on bone (Zhu et al. 2008). In elderly vitamin D deficient women, the

400 IU of vitamin D_3 and 500mg of calcium supplementation increased BMD at the lumbar spine, femoral region and in total body (Grados et al. 2003). On the other hand, weekly 10000 IU of vitamin D_2 did not have any effect over 1000mg of calcium alone on the early postmenopausal BMD (Cooper et al. 2003). The discrepancies in these results highlight the problems with different vitamin D dosages, intervals between dosing (Chel et al. 2008), different forms of vitamin D (Richy et al. 2005) and variations in study populations. Even the vitamin D receptor genotype has been associated with the effect of vitamin D supplementation on the BMD of the femoral neck (Graafmans et al. 1997).

An earlier study has shown that calcium supplementation has only a minor effect on the loss of cortical bone and no effect on the trabecular bone (Riis et al. 1987). In another study, vitamin D supplementation has also been associated with reduced seasonal bone loss at the spine, which is mostly trabecular bone (Dawson-Hughes et al. 1991). Furthermore, vitamin D and calcium supplementation have decreased the seasonal change in both the spinal and femoral BMD (Meier et al. 2004). The age-related decrease in cortical bone density and geometry has been reported to lead to decreased bone biomechanical properties (Ito et al. 2010). Accordingly, the present finding that vitamin D and calcium supplementation affected total body is important since most of the skeleton is constituted of cortical bone.

The rate of bone loss in total hip has been reported to be 0.025g/cm2 per year in women in the age group 67-69 years old (Ensrud et al. 1995). In this study, the bone loss in the total hip was slightly less, 0.014g/cm2 per three years in both groups. At the beginning of menopause, the annual bone loss at the femoral neck has been shown to be as high as -1.8% decreasing to -0.5% for women who are more than five years after menopause (Pouilles et al. 1995). Another

study reported -0.7 to -0.8% annual bone loss rates for different femoral regions (Greenspan et al. 1994). This study detected a bone loss of around -2% over three years at the femoral neck in both groups, which is similar to many previous studies.

6.6 Vitamin D and calcium supplementation and physical capacity

There were no statistically significant changes in the physical capacity tests between the study groups during the trial. Muscle strength is known to decline with age in healthy elderly subjects with adequate vitamin D status (Boonen et al. 1998) and vitamin D has not been shown to prevent this loss of muscle strength (Grady et al. 1991). However, in vitamin D deficient elderly individuals, supplementation can have a positive effect on muscle strength (Janssen et al. 2002). A trend for better maintained regular walking speed in the supplementation group might reflect better preserved lower extremity function. However, the extension strength of the lower limbs did not differ between the groups and nor was it associated with falls. The finding that the baseline SOOF test, which measures primarily the dynamic balance, was associated with subsequent falls is support for this hypothesis. The effect of vitamin D on fall prevention has previously been claimed to be mediated through both better postural and dynamic balance (Bischoff-Ferrari et al. 2006). However, vitamin D did not improve the SOOF test performance in the present study.

Earlier studies have reported both the positive (Bischoff et al. 2003) and negative (Pramyothin et al. 2009) effects of vitamin D and calcium supplementation on musculoskeletal function. Furthermore, it has also been reported that 25(OH)D levels are not associated with

neuromuscular function (Faulkner et al. 2006). Vitamin D supplementation has been increased proximal muscle strength in adults with vitamin D deficiency but it has not significantly affected muscle strength in adults with baseline 25(OH)D level over 25nmol/l (Stockton et al. 2010). The reason why physical capacity parameters were not changed differentially between the groups in the present study might be due to the fact that younger and healthier subjects were evaluated than in previous studies. The contrast between intervention and control group may have been too small due to the open design and increased vitamin D intake in the control group. Nonetheless, there were still factors related to potential errors in the physical test measurements or in the interpretation of the results (Sirola et al. 2004). The reliability of physical tests has been shown to be good to moderate in previous studies but unfortunately they were not validated in the present study. However, specially trained personnel were recruited to minimize the measurement of uncertainties. Another explanation for the null results in the physical capacity might be that possible improvements would have been missed in these physical tests. Thus, fast-pace walking speed has been associated with 25(OH)D levels and proposed to be a more sensitive marker of neuromuscular functioning compared to usual-pace walking (Annweiler et al. 2010).

There is some evidence that the 25(OH)D level threshold for muscle strength benefit may be around 40 nmol/l (Bischoff-Ferrari et al. 2004). In addition, an 25(OH)D level below 50nmol/l has been associated with increased body sway and a level below 30nmol/l with decreased muscle strength (Pfeifer et al. 2002). If this is the case, then a possible explanation for the null result is that both groups were close to or even over these thresholds. On the other hand, there is earlier evidence that serum 25(OH)D concentrations above 90 to 100nmol/l are best for lower extremity strength (Bischoff-Ferrari et al. 2004) and above 75nmol/l for fall prevention

(Bischoff-Ferrari et al. 2007). In the present study, the intervention group reached the mean 25(OH)D level of 74.6nmol/l and accordingly it could explain the decreased incidence of multiple falls. However, serum 25(OH)D did not reach a desirable level for the best muscle strength benefits. Thus, the weak effect and the negative finding in the entire trial population may also be partly due to the low number of severely vitamin D deficient women in this ambulatory postmenopausal population.

6.7 Effect of supplementation on serum 25(OH)D levels and adverse effects

The serum 25(OH)D levels rose 24.7 (24.1) nmol/l and 6.8 (19.3) nmol/l in the intervention and control groups, respectively. At the end of the trial, the corresponding mean 25(OH)D levels were 74.6 (21.9) nmol/l and 55.9 (21.8) nmol/l. As discussed above, a serum level above 75nmol/l has been claimed to be effective for fall prevention and 90-100nmol/l for adequate lower extremity strength. However, even these levels might be not sufficient for fracture prevention (Salovaara et al. 2010).

The points discussed above; younger age, healthier study population, incomplete compliance and increased supplementation use in the control group as well as relatively high nutritional calcium and vitamin D intake, might have affected the achieved serum 25(OH)D levels. It should be remembered that over 90% of vitamin D is derived from ultraviolet B light (Pearce et al. 2010) and in Finland for over half a year the amounts of sunshine are insufficient for proper vitamin D synthesis. The season variation in serum 25(OH)D levels was assessed by taking the blood samples in visits that were distributed throughout the calendar year.

In OSTPRE-FPS, only 113 out of 1585 women discontinued the intervention due to adverse effects. The most common adverse effects were gastrointestinal symptoms, nausea and skin reactions. Accordingly, there were not any severe side effects associated with the vitamin D and calcium supplementation.

7 CLINICAL RECOMMENDATIONS AND CONCLUSIONS

The first aim of the present study was to determine whether relatively simple and clinically applicable physical tests could be useful in prediction of bone density in postmenopausal women. It is proposed that grip strength could be used in medical decision making to identify those women who would benefit from BMD measurements, albeit alone it may not represent an accurate enough tool for osteoporosis screening.

Poor functional status has appeared to associate with injurious falls and consequent fractures. The second aim was to define association between functional capacity and fractures. According to the results, the standing-on-one-foot less than 10 seconds, grip strength and a question about ability to walk less than 100 meters may help to predict postmenopausal fractures.

Vitamin D deficiency is common in the elderly, and it has been associated with many health problems. The third and fourth aims were to determine if vitamin D and calcium supplementation could prevent falls and bone loss in ambulatory general population of postmenopausal women. Overall, the primary analysis revealed no association between calcium and vitamin D supplementation and risk of falls. However, the results of a post hoc analysis indicated that the supplementation might decrease the risk of multiple falls requiring medical attention. Furthermore, the results indicated that daily vitamin D and calcium supplementation have a positive effect on the skeleton in ambulatory postmenopausal women

with adequate nutritional calcium intake without any severe side effects. In conclusion, higher vitamin D intake can be recommended for the general population of postmenopausal women.

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Matti Kärkkäinen

Physical Capacity and Supplementation of Vitamin D and Calcium in Postmenopausal Women

In general, the fracture risk is highest among those who have osteoporotic bone mineral density (BMD) but nonetheless most fragility fractures occur in subjects who do not have osteoporosis. Their poor physical condition might predispose these individuals both to low BMD and a higher risk for fall-related fractures. Vitamin D has been shown to have also physiological importance outside of bone health and calcium homeostasis, and there is evidence that it plays a role in the prevention of falling.

The present thesis was based on Kuopio Osteoporosis Risk Factor and Prevention study (OSTPRE) and its substudy OSTPRE Fracture Prevention Study (OSTPRE-FPS).

UNIVERSITY OF EASTERN FINLAND

Publications of the University of Eastern Finland
Dissertations in Health Sciences

ISBN 978-952-61-0309-9