Characteristics of hip fracture cases among community-dwelling Icelandic older adults

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Thesis for the degree of Philosophiae Doctor

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UNIVERSITY OF ICELAND
SCHOOL OF HEALTH SCIENCES
FACULTY OF FOOD SCIENCE AND NUTRITION

Let us never consider ourselves finished nurses...we must be learning all of our lives. Florence Nightingale

Einkenni mjaðmabrotahóps meðal eldri einstaklinga í sjálfstæðri búsetu

Sigrún Sunna Skúladóttir

Ritgerð til doktorsgráðu

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Ágrip

Bakgrunnur: Mjaðmabrot fela í sér verulega skerðingu á heilsu og lífsgæðum. Orsakaþættir mjaðmabrota meðal aldraða eru m.a aukið tap á beinmassa, hreyfifærni og vöðvamassa með hækkandi aldri. Tengsl D vítamíns búskaps við styrk beina og mjaðmabrota hafa verið skoðuð ítarlega í fyrri rannsóknum. Þau tengsl virðast oft sterkari en búast mætti við út frá tengslum D vítamíns við beinþéttni. Færri rannsóknir hafa hins vegar verið gerðar sem skoða tengsl hreyfigetu við mjaðmabrot. Þ.e.a.s. færni sem að hluta til er hægt að hafa áhrif á með réttu mataræði og hreyfingu.

Markmið: Að kortleggja hvað einkennir aldraða einstaklinga sem mjaðmabrotna frá þeim sem brotna ekki með áherslu á að skoða áhættuþætti sem tengjast skertri beinþéttni annars vegar og skertri hreyfigetu hins vegar; og meta hvernig þessir þættir tengjast D vítamín hag þátttakenda.

Aðferðir: Notast var við gögn úr Öldrunarrannsókn Hjartaverndar (AGES-Reykjavík) sem samanstendur af 5764 þátttakendum sem skráðir voru í rannsóknina á árunum 2002 til 2006. Umfangsmiklum upplýsingum um heilsu og lífstíl þátttakenda var safnað við skráningu; þar á meðal mælingum á beinþéttni og mælingum á D vítamínhag í blóði, ásamt öðrum lífmerkjum. Upplýsingum um mjaðmabrot til loka árs 2012 var safnað úr sjúkraskrám.

Niðurstöður: Grein 1: Einkenni þátttakenda í Öldrunarrannsókn Hjartaverndar við upphaf rannsóknar (2002-2006) voru skoðuð með m.t.t. líkamssamsetningar, mælinga á hreyfifærni, styrkleika, D vítamín hag í blóði ásamt öðrum lífmerkjum. Þær mælingar voru bornar saman milli þátttakenda sem mjaðmabrotnuðu eða brotnuðu ekki á meðan eftrirfylgni stóð (til loka 2012). Það sem einkenndi karlmenn sem brotnuðu var marktæk skert hreyfigeta við upphaf rannsóknar sem kom fram í verri gildum við mælingum á jafnvægi og hraða í svokölluðu “Timed Up and Go“ prófi. Einnig var styrkur í lærlegg minni meðal karlmanna sem brotnuðu. Þessi munur var greinilegur þó leiðrétt væri fyrir bæði aldri og beinþéttni við upphaf rannsóknar. Það sem einkennir konur sem mjaðmabrotna var marktækt lægri líkamsþyngdarstuðull, minni fitu- og vöðvamassi ásamt því að minni styrkur mældist í lærlegg. Styrkur D vítamíns í blóði (serum 25(OH)D) var marktækt lægri (~5 nmol/L) við upphaf rannsóknar hjá báðum kynjum sem brotnuðu.

Grein 2: Hér voru tengsl D vítamín hags við upphaf rannsóknar skoðuð nánar með tilliti til beinþéttni annars vegar og mjaðmabrota hins vega ásamt því að taka þætti sem mæla hreyfigetu til greina. Eftir að hafa leiðrétt fyrir bæði aldri og líkamsþyngdarstuðli þá var ófullnægjandi D vítamín hagur í blóði við upphaf rannsóknar (<30 nmól/L) borið saman við þá sem voru með fullnægjandi hag (≥50 nmól/L) marktækt tengdur lægri beinþéttni í lærleggs hálsi (femoral neck) hjá bæði körlum -17 mg/cm^3 (95% öryggisbil (CI): -26, -8) og konum -7 mg/cm^3 (95%CI: -14, -1). Lélegur D vítamín hagur var einnig marktækt tengdur auknum líkum á mjaðmabrotum á meðan eftirfylgni stóð hjá bæði körlum [Hazard ratio (HR): 3.1 (95%CI: 1,9, 5.2)] og konum [1.8 (95%CI: 1.3, 2.5)] borið saman við þá sem voru með fullnægjandi hag. Ef leiðrétt var fyrir bæði beinþéttni við upphaf rannsóknar og þáttum sem endurspegla hreyfigetu hurfu þessi tengsl hjá körlum [HR: 1.3 (95%CI: 0.6, 2.5)] en ekki konum [HR: 1.7 (1.1, 2.4)]. Frekari greiningar bentu til þess að lægri beinmassi hjá konum tengdur minna rúmmáli beina gæti að hluta útskýrt af hverju hreyfigeta og beinþéttni hafði minna vægi hjá konum en körlum.

Grein 3: Hér voru tengsl mjólkurneyslu þátttakenda við upphaf rannsóknar og mjaðmabrota skoðuð. Í ljós kom að þeir sem borðuðu minna en hálfan skammt (einn skammtur ~250 mL) af mjólk eða sýrðum mjólkurvörum á dag voru í töluvert meiri áhættu á mjaðmabrotum miðað við þá sem borðuð tvo eða fleiri skammta á dag [HR 1.46 (95%CI 1.01, 2.11)], sem er sá skammtur sem opinberar ráðleggingar leggja til varðandi mjólkurneyslu.

Ályktanir: Töluverður kynjamunur virðist vera á einkennum þeirra sem mjaðmabrotna í Öldrunarrannsókn Hjartaverndar þar sem líkamssamsetning hjá konum virðist hafa meira vægi meðan mælingar sem endurspegla hreyfigetu hafa meira vægi hjá körlum. Skýr tengsl fundust milli ófullnægjandi D vítamín hags og mjólkurneyslu undir ráðleggingum (≥2 skammtar á dag) við aukinna áhættu á mjaðmabrotum hjá báðum kynjum þó hugsanleg orsakatengsl gætu verið mismunandi milli kynja. Þessar niðurstöður benda til þess að fylgni við fæðutengda ráðleggingar gæti haft fyrirbyggjandi áhrif þegar kemur að áhættu á mjaðmabrotum meðal aldraða.

Lykilorð:

Mjaðmabrot, aldraðir, beinþéttni, 25(OH)D, líkamleg virkni.

Abstract

Background: Hip fractures in older adults are strongly associated with reduced health and quality of life. Loss of bone mass, physical function and muscle mass are thought to be main causes of hip fractures at older age. Associations between vitamin D status and bone mineral density (BMD) and hip-fractures have also been extensively explored in epidemiological studies. The observed strength for the association between vitamin D status and hip fractures often appears stronger than what would be expected by associations reported between vitamin D status and BMD alone. Fewer studies have, however, examined the possible role of factors reflecting physical function and mobility at older age in relation to hip-fractures. That is, factors that may be modifiable through healthy diet and physical exercise.

Aims: To characterize differences between older adults that experience hip fractures compared to those who do not, with an emphasis on measures of bone mass on one hand and physical function on the other. Furthermore, to determine to what extent measures of volumetric BMD (vBMD) of the femoral neck and physical function may be associated with vitamin D status.

Results: In paper-I baseline (2002-2006) characteristics of those who experienced hip fractures over the follow-up period (until 2012) versus those who did not were compared. Males that experienced hip fractures had significantly poorer measures of physical function, including performance in balance test and the time up and go test compared to males that did not experience hip fracture. These differences were still significant after adjustment for age and vBMD. Similar differences were not observed in females. In contrast, female hip fracture cases had lower body mass index, fat- and fat free mass compared to those that did not experience hip fractures. However, similarities between both sexes included lower vitamin D status (~5 nmol/L lower concentration of serum 25(OH)D), and lower leg strength among hip fracture cases compared to non-cases.

In paper II the association between vitamin D status and vBMD at baseline as well as incident hip fractures were explored, taking measures of physical function into consideration as possible explanatory factors. After adjustment for age and BMI, deficient (serum 25(OH)D concentrations <30 nmol/L) compared to sufficient status (≥50 nmol/L) vitamin D was associated with lower vBMD of the femoral neck at baseline in both males -17 mg/cm^3

(95% confidence interval (CI): -26, -8) and females -7 mg/cm^3 (95%CI: -14, -1). Deficient versus sufficient vitamin D status was also significantly associated with higher risk of incident hip fractures in males [Hazard ratio (HR): 3.1 (95%CI: 1,9, 5.2)] and females [1.8 (95%CI: 1.3, 2.5)]. After further adjustment for vBMD and measures of physical function the association became non-significant for males [HR: 1.3 (95%CI: 0.6, 2.5)] but remained significant for females [HR: 1.7 (1.1, 2.4)]. Further analyses suggested that these sex specific differences might relate to lower bone mass among females due to smaller bone volume that made the adjustment of vBMD and physical function less influential for females.

In the third and final paper, the association between consumption of milk and cultured milk products with incident hip fractures was explored. Those participants who consumed less than half a portion of milk products per day (one portion ~250 mL) had significantly higher risk of incident hip fractures compared to those consuming ≥2 portions per day [HR 1.46 (95%CI 1.01, 2.11)], which is the amount that official dietary recommendations advocate.

Conclusions: Sex specific differences were observed between hip fracture cases and non-cases. Measures of physical function and balance were more pronounced for males, while measures of body composition were more pronounced for females. Vitamin D status and consumption of milk and cultured milk products showed a relatively strong and protective association with incident hip fractures in Icelandic older adults. The possible causes for these observed associations appeared to differ between males and females.

Keywords:

Hip fracture, aging, bone mineral density, serum 25(OH)D and physical function.

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Contents

List of abbreviations

AGES-Reykjavik study: Age, Gene/Environment Susceptibility-Reykjavik Study

vBMD: Volumetric bone mineral density

BMD: Bone mineral density

BMC: Bone mineral content

BV: Bone volume

BMI: Body mass index

CI: Confidence interval

CCI: Charlson comorbidity index

CT: Computed tomography

DEXA: Dual X-ray absorptiometry scan

HR: Hazard ratio

ICD: International Statistical Classification of Diseases and Related Health Problems

IHA: Icelandic Heart Association

NNR: Nordic nutrition recommendations

TUG: Timed up and Go test

WHO: World Health Organization

QCT: Quantitative computed tomography

25(OH)D: 25-hydroxy vitamin D from serum blood.

List of figures

List of tables

List of original papers

This thesis is based on the following original publications, which are referred to in the text by their Roman numerals I-III. The first two papers are reprinted in chapter by kind permission of the publishers.

I. Skuladottir SS, Ramel A, Hjaltadottir I, et al. (2020). Characteristics of incident hip fracture cases in older adults participating in the longitudinal AGES-Reykjavik study. *Osteoporosis International*

II. Skuladottir SS, Ramel A, Hjaltadottir I, et al. (2021). Serum 25-Hydroxy-Vitamin D Status and Incident Hip Fractures in Elderly Adults: Looking Beyond Bone Mineral Density. J Bone Miner Res. 2021 Dec;36(12):2351-2360

III. Skuladottir SS, Hjaltadottir I, Siggeirsdottir K. et al. (2022), Milk intake and hip fracture incidence in community-dwelling old Icelandic adults. (Manuscript in review)

Declaration of contribution

Paper-I: Study concept and design*: Sigrún Sunna Skúladóttir* (SSS). *Laufey Steingrímsdóttir* (LS), *Gunnar Sigurðsson* (GS) and *Thorhallur Ingi Halldorsson* (TIH). Acquisitions of data: VG, GS and KS. Analysis and interpretation SSS and TIH. Drafting of the manuscript: SSS and TIH. Critical revision of the manuscript SSS, TIH, LS, GS, *Mary Frances Cotch* (M-F C), *Lenore J Launer* (LJL), *Vilmundur Gudnason* (VG), *Ingibjorg Hjaltadottir* (IH) and *Kristin Siggeirsdottir* (KS). Statistical analysis SSS and TIH.

Paper-II: Study concept and design: SSS, *Alfons Ramel* (AR), GS and TIH. Acquisitions of the data: SSS and TIH. Acquisitions of data: VG, GS and KS. Analysis and interpretation SSS and TIH. Drafting of the manuscript: SSS, AR and TIH. Critical revision of the manuscript SSS, AR, TIH, LS, GS, M-F C, LJL, VG, IH and KS. Statistical analysis SSS, AR and TIH.

Paper-III: Study concept and design: SSS, AR, GS and TIH. Acquisitions of data: VG, GS and KS. Analysis and interpretation SSS AR and TIH. Drafting of the manuscript: SSS, AR and TIH. Critical revision of the manuscript SSS, AR, TIH, LS, GS, M-F C, LL, VG, IH and KS. Statistical analysis SSS, AR and TIH.

AR, TIH and SSS had full access to all of the data in the studies and take responsibility for the integrity of the data and the accuracy of the data analysis.

1 Introduction

This dissertation aims to expand the knowledge of the risk of hip fracture in older adults by examining what characteristics hip fracture cases compared to non-cases using the rich source of data collected in the Age, Gene/Environment Susceptibility-Reykjavik Study (AGES-Reykjavik study).

The National Institutes of Health (NIH) defines hip fractures as ”*cracks or breaks in the top of the thigh bone (femur) close to the hip joint, usually caused by a fall or an injury to the side of the hip*”[1]. Falls from a standing height are the cause >90% of hip fractures [2-4]. According to the ICD 10th classification, hip fractures are divided into three groups: S72.0 fractures in the neck of the femur, S72.1 fractures of the trochanter major, and S72.2 fracture on the trochanter minor or fractures occurring up to 5 cm below trochanter minor [5].

Hip fracture rates differ markedly by sexes, with around 70% of all fractures occurring in females [6-8]. It has been suggested that the reason for this higher rate among females is related to accelerated bone loss due to hormonal changes associated with menopause [9, 10]. Age is one of the strongest risk determinants identified for hip fractures [11]. According to studies from both Europe and Asia, the mean age (± standard deviation) for hip fractures is around 77±7 years [12-16]. This estimate corresponds well with results from other individual studies conducted in various countries including Sweden, the Netherlands and Iceland where the mean age ranges reported were between 80±5 to 82±8 years [17-20].

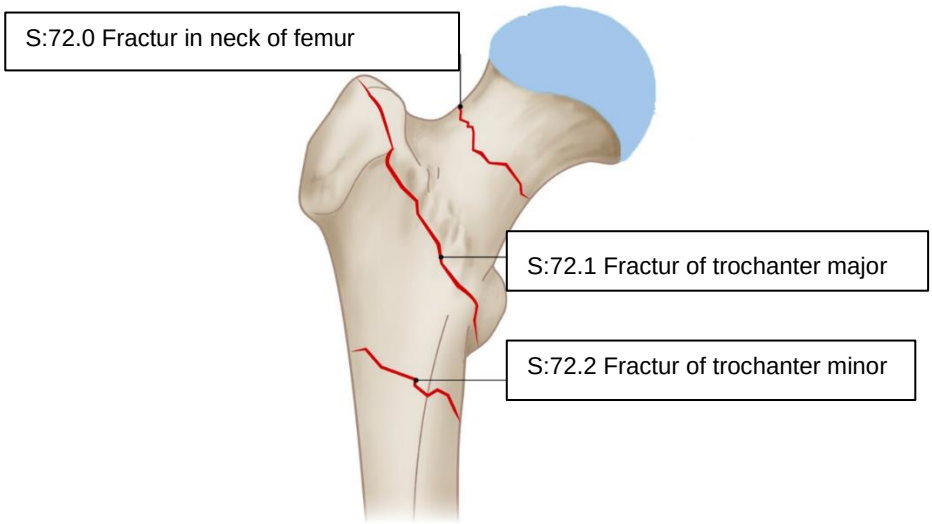

Figure 1. The Classification of hip fractures on femur, according to ICD-10.

It has been predicted that incidence of hip fractures will continue to increase as the older adult population grows due to increased life expectancy [21]. However, this prediction is currently not entirely consistent with population time trend studies. For example, a study from Denmark covering a period of over 35 years reported a slight decrease in incidence from 5.4 per 1000 person-years in 1980 to 4.2 per 1000 person-years in 2014 [22]. However, an Australian population-based cohort study reported a 9% increase in incidence between 2000 to 2012 [23]. Similarly, a study from Spain reported a 4% annual increase in incidence from 1997 to 2010 [24]. The comparability of these estimates is subject to some uncertainty as it is unclear from some of these studies if age was accounted for.

There is also a large geographical variation in the incidence of hip fracture. The highest rate of hip fractures worldwide has been reported in studies from Scandinavia while the lowest have been reported in Africa [25]. For instance, a systematic review on hip fracture incidences reported 57 cases per 10.000 individuals for females and 29 cases for males. Similar estimates have been reported from Norway [26] and Iceland [18] as well. However, a study from Rabat, Morocco showed a rate of incidence of 5.2 for hip fractures per 10.000 persons for females and 4.4/10.000 persons for males [27] These large differences in incidence could perhaps at least partly

be explained by the fact that not all hip fracture cases in poorer regions may be treated in hospitals, leading to an underreporting of cases [28].

Hip fractures are associated with several morbidities, with those afflicted by them often becoming less independent, requiring substantial assistance with daily activities [29-31] and experiencing a reduced quality of life [32]. Reduced quality of life is, for example, reflected by impaired ability to walk, chronic pain resulting in psychological stress and depression [32]. Studies have shown that mortality rates also increase substantially after hip fractures. For example, a longitudinal study from Sweden spanning over two decades showed a significantly higher mortality rate among those experiencing hip fractures, with slightly higher mortality rates observed for males (Hazard Ratio (HR) 5.7) compared to females (HR 4.6) [33]. Nevertheless, as many health conditions occur in older age with increased frequency and those factors may have blurring effects. In other words, the estimates for high mortality rate associated with hip fractures might be overestimated due to other confounding factors. Regardless, the high mortality rate associated with hip fractures highlights the importance of studying preventive measures.

In summary, experiencing hip fractures is strongly associated with higher mortality and morbidity, resulting in reduced quality of life. With many known risk factors such as age and sex being non-modifiable, it is important to identify and characterize modifiable risk factors that may help to reduce the burden of fractures in older adults.

1.1 Bone structure and strength

Osteoblasts and osteoclasts are two cell types that determine bone structure, with other cell types such as osteocytes and bone lining cells playing a different role [34]. Osteoblast cells are located mainly in the periosteum of the bones, synthesizing the bone matrix and maintaining the bone´s structural integrity. The osteoclast´s role is to degrade the bone to instruct normal bone remodeling and mediate bone loss in pathologic conditions by increasing their resorptive activity. Osteoblast cells regulate both the activity of bone formation and the amount of the bone´s resorption of osteoclasts. This balance between osteoblasts and osteoclasts regulates the bones' release and absorption of calcium and phosphate. If this balance is disturbed, it can influence the pathogenetic mechanism in the development of bone disease [35]. The balance between osteoblasts and osteoclasts strongly influences the absolute bone mineral content (or bone strength).

Volumetric Bone mineral density (vBMD) is a measure of the amount of bone mass per bone unit volume. That is, the vBMD is quantified based on how high the absolute bone mineral content (BMC) is for a given cross-sectional of certain thickness in the bone. These measures (bone volume, BMC and vBMD) are usually quantified using either dual X-ray absorptiometry scan (DEXA) or quantitative computed tomography (QCT). The absolute bone strength is therefore determined by the absolute bone mass (vBMD x bone volume). The absolute bone volume is an important determinant of bone strength as relatively low vBMD combined with larger bone volume may result in a higher absolute bone mass compared to slightly higher vBMD in a smaller bone volume.

Osteoporosis is defined by the World Health Organisation (WHO) as having BMD that is at or below 2.5 SD of the average for healthy young adults [36]. Osteopenia is similarly defined as having BMD that is between 1 to 2.5 standard deviations lower than the average for healthy young adults [37]. It is worth noting that the higher prevalence of osteoporosis and osteopenia among post-menopausal females due to associated hormonal changes, is perhaps one reason why prevalence of hip fractures is consistently observed higher in females compared to males [38, 39]. Another important explanation for higher prevalence among females might be related to their smaller bone volume, which to a large extent determines absolute bone mass (or strength) [40].

Clinical prediction models have been set up to quantify the risk of possible future hip fractures based on established risk factors on an individual basis. One such prediction model is *the Fracture Risk Assessment Tool (FRAX)*, which is a widely used tool in clinical settings that predicts individual risk for fractures over the next 10 years. The risk calculation is based on information such as age, height, weight, sex, history of arthritis, osteoporosis and previous fractures. Other factors such as family history of fractures, alcohol use, smoking habits, and medication use (e.g., glucocorticoids) are also taken into consideration [41]. The use of BMD in the risk calculation is, however, only optional. As such, measures are available that can be used for making more accurate predictions. The FRAX algorithm has been criticized for not taking information on nutritional factors that may influence bone strength into account, such as calcium and Vitamin D-vitamin status [42]. Information on physical function (e.g., leg strength, balance or other functional measures) which are also well established risk factors for predisposition to falling are not included either [42].

1.2 Factors that determine the propensity of falls

Almost all hip fractures occur after a fall, and the majority at up to 90%, are low energy traumas occurring after falling from a standing height [3, 4, 43] . Few hip fracture cases occur after high energy trauma, usually among young people who fall from a height more than two times their own height, or other high-impact occurrences such as car crashes.

High propensity for falls may indicate a patient's poor physical function, balance, or muscular strength, and results from functional tests indicating poor balance and strength of the leg and arm, or Timed Up and Go (TUG) test, have been associated with a higher risk of falling [44, 45]. Other factors associated with increased risk of falling include polypharmacy (e.g. simultaneous use of many medications) [46-48]. Much less attention has been given to risk factors for falls in studies focusing on BMD and osteoporosis, which are often modifiable and may play a major role in prevention, such as in the case of physical function.

1.2.1 Frailty and sarcopenia

Scientific interest in frailty in geriatrics has been increasing within the last few years [49]. A search at Pubmed.gov on "frailty and geriatrics" gave 112 related results between the years 1900 to 2000 and 9110 results between the years 2000 to 2022. Similarly, publications on frailty and postoperative outcomes in older surgical patients in a systematic review from 2016 reported a fivefold increase since 2010 [50]. Studies have associated frailty in old adults with higher risks of mortality and complications during hospital treatment as well as longer periods of hospitalization following surgery [50]. Frailty has also been associated with increased risk of fracture [51].

Frailty is mainly defined based on two commonly used approaches. The first approach is called the *'phenotype' model*, as proposed by Fried et al. in his publication from 2001 [52]. The *Phenotype model* is based on markers of frailty such as weight loss, muscular weakness, grip strength, exhaustion, slowness measured by gait speed, and physical activity [52]. The other commonly used approach is called the *Frailty Index* (or *cumulative deficit model*), which was developed based on the findings from the Canadian Study of Health and Aging [53]. Rockwood and colleagues continued working on the *Frailty Index*, with the aim to develop a more practical scale for clinicians, and the *CSHA Clinical Frailty Scale*, is their scale for clinical use [54],

The term sarcopenia comes from the Greek words "sarx" meaning flesh and "penia" meaning loss. Sarcopenia is usually defined as the loss of

skeletal muscle mass and strength that occurs due to aging [55]. However, the European Working Group of Sarcopenia in Older People (EWGSOP) has highlighted that there is still no single approach to diagnosing sarcopenia that is consistently used in both research and clinical practice. EWGSOP recommends using both low muscle mass and low muscle function, as measured by strength and performance, as symptoms for diagnosing sarcopenia [56]. Their recommendation is to identify progressive and comprehensive skeletal muscle disordering through functional tests such as Timed-Up and Go, grip-strength and gait speed; and, preferably, if possible, to use measures that can directly quantify muscle mass such as dual-energy X-ray absorptiometry (Dexa) [57].

Apart from natural aging, underlying disease and medical conditions, such as malnutrition and cachexia, are strong determinants of sarcopenia [58]. Optimal nutritional status and physical activity may, however, delay the progression of sarcopenia [59-61] by preventing loss of muscle mass and strength.

Frailty and sarcopenia are strongly related conditions. A state of frailty is defined by exhaustion, weakness, and slowness, whereas the definition of sarcopenia is more focused on loss of muscle mass and strength [62]. Sarcopenia and frailty have both been strongly associated with increased propensity for falls [63] and risk of hip fractures [64]. One plausible chain of events for this relationship is demonstrated in Figure 2. As shown, advanced age is associated with higher risk of developing sarcopenia due to natural breakdown of muscle mass, which is accelerated by physical inactivity and poor nutrition. The presence of sarcopenia results in higher frailty and the associated functional impairment results in increased propensity for falls and thereby hip fractures [65]. Although studies on frailty and sarcopenia have been increasing in past years, few of them have focused on preventing or lowering risk of hip fractures, making this a gap of knowledge in the field.

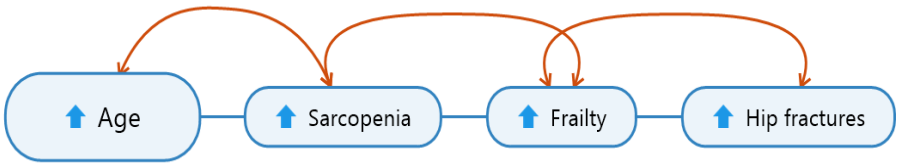

Figure 2. Conceptual presentation of aging and hip fracture risk mediated through sarcopenia and frailty.

1.3 Preventive measures for hip fractures

Preventive measures can greatly reduce the risk of hip fractures in older adults. A review on observational and randomized control studies in subjects older than 40 years of age showed that primary leisure time activity, or moderate to vigorous physical activity, reduced risk fractures including hip fractures specifically, from 1% to 40% depending on the level of activity [66]. However, exercise alone may not be sufficient, particularly in more elderly demographics, where sub-optimal nutrition and/or malnutrition is common, which can severely affect physical function [71]. It is, for example, well established that low BMI in older adults is strongly associated with higher risk of hip fractures [67, 68]. This suggests that optimal nutrition may be a crucial modifiable risk factor for prevention. Other well-known nutritional factors, which have been extensively studied in relation to bone health and risk of fractures, are vitamin D and calcium intake. Although the relationship between sufficient vitamin D levels and BMD is relatively well established [69, 70], systematic reviews on vitamin D supplementation alone or in combination with calcium have provided conflicting results, with some suggesting benefits [71] and others remaining inconclusive [72, 73]. Below is a short overview of current knowledge of possible preventive factors for hip fractures including physical activity and nutritional factors.

1.3.1 Calcium

Calcium is a mineral mainly stored in bones and teeth. Apart from its role in supporting the structure and durability of bones and teeth, calcium plays an essential role in contraction and dilation of veins as well as muscle function.

Currently established dietary recommendations for calcium are relatively similar across international borders. For example, the National Institutes of Health (NIH) recommends 1000 and 1200 mg per day for males and females aged 51-70, respectively; and 1200 mg/d for males and females >70 years [74]. Similarly the dietary reference value set by the European Food Safety Authority for adults 25 years and older is 950 mg per day [75], while the Nordic Nutrition Recommendations from 2012 set the recommended intake at 800 mg per day [76].

There are substantial regional differences in terms of average daily calcium intake with many Asian populations having an intake of less than 500 mg per day while northern European countries often have intakes greater than 1000 mg per day [77]. The main dietary sources of calcium include dairy as well as non-dairy sources such as dark green vegetables (e.g. broccoli

and spinach), nuts; and small fishes whose bones are consumed (e.g., sardines) [75]. Insufficient dietary intake of calcium, such as among those adhering to plant-based diets, can be compensated by use of calcium supplements.

Bone strength and rebuilding of bones depends on the balance between calcium intake and vitamin D status [78] with both nutrients playing a key role in preventing loss of bone mineral density [79]. Sufficient vitamin D status ensures proper calcium absorption in the small intestine, which is critical for maintaining calcium balance [78]. Release and absorption of calcium in bones is also regulated by vitamin D status and the parathyroid hormone (PTH). The PTH regulates blood calcium levels by stimulating the release of calcium from bones into the bloodstream and reducing the loss of calcium in urine [80]. PTH and Vitamin D also form a tightly controlled feedback loop with PTH stimulating vitamin D synthesis in the kidney, as shown by increased synthesis and PTH level subjects with vitamin D deficiency [81]. Depletion of calcium from the bone can also occur though other factors, such as lowering estrogen levels in females following menopause, insufficient calcium intake or during illness [80].

In the Nordic countries, including Iceland, consumption of dairy is relatively high compared to other regions and it is one of the main sources of calcium intake [76]. Information published in 2022 on calcium intake from dairy consumption in Icelanders showed that 72% of males and 46% of females reached the recommended intake of 800 mg of calcium per day [82].

Existing evidence from prospective observational studies regarding the role of dairy consumption in preventing hip fracture are somewhat conflicting. For example, a systematic review and meta-analyses of the correlation between dairy consumption and hip fractures from 2020 including studies from both Europe and North-America (n~230.000 subjects aged ≥65 years) did not find any clear reduction in risk of incident hip fractures when comparing high and low levels of consumption of various dairy products [Hazard ratio of 0.95 (95%CI: 0.87, 1.03)] [83]. However, when observing variance among individual categories of dairy products, slight statistical difference indicating increased protection were reported for yogurt [HR: 0.89 (95%CI 0.81, 0.98)] and cheese [HR: 0.92 (95%CI: 0.87, 0.98)] but not milk [HR: 1.05 (95%CI: 0.94, 1.18)]. These findings are largely consistent with conclusions from other meta-analyses [84].

Results of intervention studies examining the preventive effect of calcium supplementation on bone health are also somewhat inconsistent. For

example, a meta-analysis of randomized 15 trials (n~2000) of calcium levels in post-menopausal females (dose range across studies of 500 to 2000 mg) showed a small but significant reduction in bone loss over two years among those taking calcium supplementation relative to controls [85]. One major limitation of this analysis was that several of the included trials used a combination of calcium and vitamin D as an intervention, instead of calcium supplementation alone. On the other hand, a systematic review of longitudinal cohort studies found no association between calcium intake and BMD changes in females >60 years. However, in those studies, intake of calcium was in most cases below 500 mg/day [86], which is only half of the recommended intake [75, 76]. Another meta-analysis of prospective cohort studies from 2015 showed that low calcium intake was not associated with hip fracture risk [87].

Although evidence in favor of elderly people's high consumption of calcium-rich foods, such as dairy, bolstering their bone health is somewhat inconclusive, the importance of adequate calcium and vitamin D intake is proven by slight statistical variances, and does appear to be an important influencing factor [88].

1.3.2 Vitamin D

Vitamin D is often called "the sunshine vitamin" as one of its main sources is sunlight exposure (not just dietary intake). Vitamin D is synthesized from cholesterol like many other steroid hormones and is therefore categorized as one. Figure 3 shows vitamin D´s metabolism and its physiological role in calcium homeostasis schematically. Vitamin D´s mechanism of action follows a rather complex pathway involving synthesis in the skin or gastrointestinal absorption (after oral intake) followed by metabolism to its active form [88]. After intestinal absorption or synthesis in the skin, the biologically inactive precursor of vitamin D (cholecalciferol) is transported to the liver, where it undergoes an enzymatic hydroxylation reaction mediated by the 25-hydroxylase which forms 25-hydroxy vitamin D (25(OH)D). After the release of 25(OH)D into the bloodstream, it is transported to the kidneys, where 25-hydroxy vitamin D undergoes a second hydroxylation mediated by 1α-hydroxylase, which converts 25(OH)D to the biologically active hormone, calcitriol (or 1,25-dihydroxyvitamin D) [89]. The 1,25 dihydroxy vitamin D promotes the gut to absorb calcium. Low calcium in the blood increases the hormone parathyroid triggering the osteoclast in the bone to break down further within the bone and release more calcium into the blood. There is a balance of the circulating parathyroid hormone, which releases calcium from

the skeleton [90]. The half-life of 1,25(OH)2D r is 12 -16 hours, while the half-life of the inactive form, 25(OH)D, is about three weeks.

Given its relatively long half-life of serum 25(OH)D, vitamin D status is defined on the basis of this biomarker and its function in skeletal health. The Institute Of Medicine (IOM) on vitamin D deficient status is as follows [89, 91]:

- deficiency as serum 25(OH)D <30 nmol/L,
- inadequacy as serum 25(OH)D ≥30 to <50 nmol/L
- sufficiency as serum 25(OH)D ≥50 nmol/L

The IOM recommendation also notes that serum 25(OH)D above 125 nmol/L and in particular above 150 nmol/L may be associated with adverse effects such as hypercalcemia.

Living in northerly latitudes such as Iceland, provides limited opportunities to gain vitamin D from the sun. Apart from a limited number of hours of sunshine, the heavier clothing that is associated with colder climates, which leaves far less skin exposed, also negatively impacts sunlight exposure. The skin of older adults is less effective in absorbing ultraviolet light for vitamin D synthesis [92]. As a result, dietary intake of vitamin D is often the main source among the elderly and people living northerly latitudes. However, relatively few food items available in these areas contain sufficient concentrations of vitamin D to meet recommended intake, which is 15 µg for <70 years and 20 µg for ≥70 years [89], with the notable exception of seafood, predominantly fatty fish [93]. Dietary supplements are the other major dietary source of vitamin D, with fish liver oil being by far the most widely used supplement in Iceland [94].

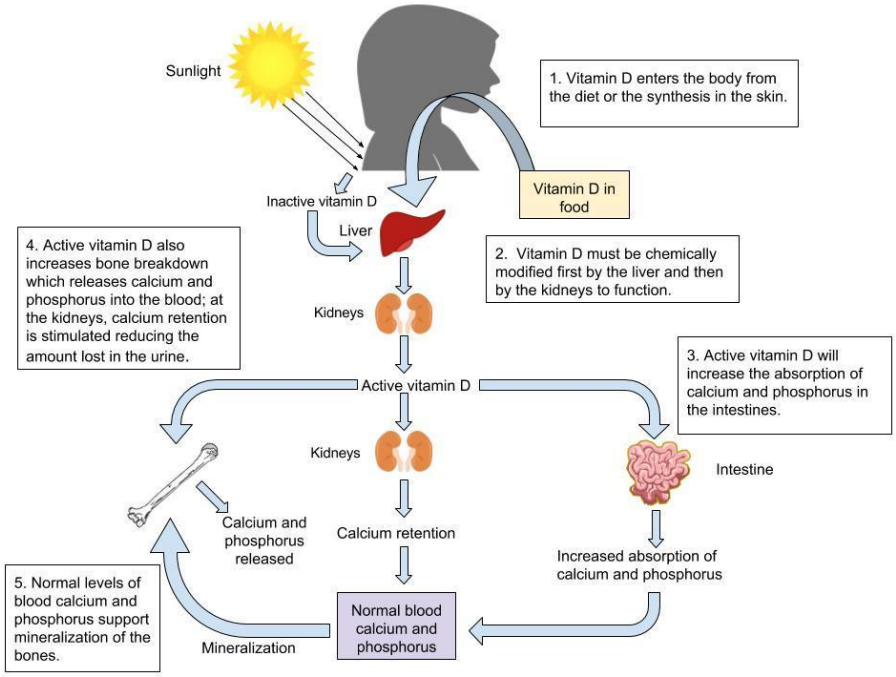

Figure 3. The figure shows a schematic outline of vitamin D metabolism and its physiological role in calcium homeostasis following dietary intake or skin synthesis [95].

1.3.3 Vitamin D biomineralization and risk of fractures

In its systematic review for setting the dietary reference value for vitamin D, the European Food Safety Authority concluded that although there is consistent evidence suggesting that vitamin D intake resulting in serum 25(OH)D levels above 50 nmol/L is sufficient to maintain bone health, evidence in support of its role as a preventive measure against fractures is less certain [93].

Despite EFSA's conclusion that evidence suggesting a causal relationship of vitamin D in preventing fracture is weak at best, it seems biologically plausible that such an effect may exist, given the role of vitamin D in calcium absorption and bone mineralization [96]. Several observational studies have reported both a positive association between 25(OH)D and bone mineral density (BMD) and a lower risk of fractures [39, 97, 98]. Furthermore, although some randomized controlled studies have shown positive effects on the rate of bone loss among the elderly [99], results from RCTs examining effects on bone mineralization have not been entirely consistent [100]. RCTs

have limitations, thus two conditions should ideally be met in any such trials. Firstly, there should be sufficient compliance among participants over a sufficient period of time for the intervention to have an effect. Secondly, participants would need to benefit from any treatment if any health effect is to be expected, which implies that participants would need to be vitamin D insufficient or deficient. These conditions are often not met in existing trials [101], which may explain inconsistencies between RTCs and observational studies on vitamin D and bone mineralization.

The evidence in support of vitamin D's effect on the risk of fractures is even less clear. The most recent Cochrane review from 2014 concluded that vitamin D supplementation alone was unlikely to prevent fractures despite possible modest beneficial effects on BMD [102]. This conclusion was based on findings from 11 trials that included 27,693 participants with a resulting relative risk (RR) estimate of 1.12 (95%CI: 0.98, 1.29). However, when examining the preventive effects of calcium and vitamin D supplementation combined, there was a significant reduction in the risk of incident hip fractures [9 trials, 49,853 participants with a RR of 0.84 (95% CI 0.74 to 0.96)]. In contrast, observational studies provide some evidence for the beneficial role of vitamin D in preventing fractures, particularly among those who are vitamin D insufficient or deficient. For example, in research conducted by the Cardiovascular Health Study of older participants with a mean age of 74 years, serum vitamin D <15 ng/mL was associated with a 61% greater risk of hip fracture. The mean follow up time was 13 years, by which time 242 participants had a hip fracture [103]. In a population-based Swedish cohort study of 61,433 females followed for 19 years, vitamin D intake of <5 µg/d was associated with an increased risk of hip fractures when compared to an intake of >10 µg/d [HR: 1.27 (95% CI 1.03-1.57)] [104]. Other longitudinal studies showed similar results [97, 105]

Although RCTs on vitamin D supplementation alone do not conclusively affirm its role in preventing fractures [102], other RCTs have shown that vitamin D supplementation seems to decrease the risk of damaging falls [106, 107]; which are, as previously mentioned, one of the most frequent causes of hip fractures. One suggested explanation for the observed effects of vitamin D supplementation on propensity to fall is a link between vitamin D status and physical function. Other RCTs supported these findings, showing some beneficial effects of vitamin D supplementation on muscle strength[108] and balance [109].

In summary, existing studies provide relatively strong evidence for the benefits of having sufficient vitamin D status [93] to prevent loss of bone mass. However, existing evidence is less clear on the preventive role of Vitamin D on fractures. Results may relate to the methodological challenges of conducting randomized controlled trials over long periods of time, which are needed for this outcome and the complex role of vitamin D on not only bone health but also possibly physical function [110].

1.3.4 Physical activity

WHO defines physical activity as "*any movement involving skeletal muscles that requires energy expenditure"* [111]. Similarly, physical function is defined as *the ability to be capable of taking care of oneself or being able to maintain independence* [112]. Physical activity is often assessed through subjective methods such as questionnaires where participants are asked about the frequency and duration of different activities. Objective measures are also possible through the use of accelerometers and other devices. In contrast, a physical function can be assessed more directly through functional tests such as TUG [107], hand and/or leg strength [113] and balance [66], which often accurately reflect a person's ability to take care of themself.

In observational studies, older adults engaging in low levels of physical activity, have generally been found to have a higher risk of hip fracture compared to those who are regularly physically active [45, 114-116]. One critical benefit of physical activity on bone health is that mechanical stress is essential for developing and maintaining muscle and bone strength through stimulation of bone remodeling [117].

Interest in the relationship between physical function and hip fractures has been growing in recent years. Such studies have shown that poorer performance in tests measuring balance, leg and grip strength, Timed Up and Go, and walking speed are associated with a higher risk of hip fracture [118-120]. Increased propensity for falls is considered an indicator of poor physical function, and has also been associated with a higher risk of hip fracture [118, 121-125]. For example, in the early EPIDOS study published in The Lancet in the late 1990s, poor balance and physical function were significantly associated with an increased risk of subsequent hip fractures, even after adjustment for bone mineral density [121]. This would suggest that the association with hip fracture was driven at least in part by other factors than bone health alone, possibly through increased propensity for falls.

One advantage of measuring physical function for preventative measures in older adults specifically is that improvements in physical function are possible through engagement in regular physical activity. Measures of physical function, including functional tests such as leg or grip strength or TUG, are simple, non-expensive tests that do not require expensive equipment in a hospital setting [126]. The extent to which successful maintenance of good physical function is successful in preventing fractures through bone strength or muscle strength is currently not well-defined in praxis, and the possible role of modifiable risk factors such as vitamin D in both physical function and bone health remains to be explored in more detail.

1.3.5 Lifestyle factors associated with hip fractures

Several lifestyle factors have been identified as predictors of hip fractures, although the pathway on which they may operate is not fully understood. A systematic review by Berg and colleagues from 2008 found that heavy alcohol users had a higher risk of hip fracture compared to those who consumed <1 drink per day [relative risk (RR) of 1.39 (95% confidence interval, 1.08-1.79) [127]. The results are nearly identical to a more recent review from Mortensen and colleges from 2021 [68]. Smoking has also been associated with a higher risk of hip fracture in patients, with a meta-analysis from Wu and colleagues (2016) estimating the RR among male smokers to be 1.47 (95% CI 1.28-1.66) [128]. Other lifestyle factors associated with a higher risk of hip fractures are: drinking >3 cups of coffee per day and having low body mass index [68].

2 Aims

The overall aim of this PhD project was to investigate the shared characteristics and habits of older Icelandic adults who experience hip fractures, and identify modifiable risk factors that could prevent them. To that end, the longitudinal AGES-Reykjavik study from the Icelandic Heart Association was used which recruited 5764 participants between 2002 to 2006. Information on incidence rates of hip fractures was extracted through hospital records until the end of 2012. This thesis consists of three interlinked papers defined by the following objectives:

- **Paper-I:** To characterize baseline characteristics of those who later experienced hip fractures in the AGES-Reykjavik study versus those who did not experience hip fractures. In these analyses, the aim was to explore risk factors that might be potentially mediated through bone health, as well as risk factors that might be mediated through physical function on other risk factors.
- **Paper-II:** One of the modifiable risks for hip fracture risk identified in paper-I was vitamin D status (or serum 25(OHD), with better vitamin D statuses being associated with lower risk of fractures. The observed association appeared to be mediated through both higher bone mass and better physical function. The aim of paper-II was to further explore how the measurements of bone mass and physical function might explain the inverse association between serum 25(OH)D and the incidence of hip fracture observed in the paper-I.
- **Paper-III**. The focus of the third and final paper was on diet as a potentially modifiable risk factor for hip fracture. More specifically, dairy is an important source of calcium and protein intake in older Icelandic adults. The objective of this paper was to examine the association between baseline intake of dairy and incidence of hip fractures. Associations between dairy intake, bone mass and physical function were also explored.

Papers I to III are provided in Appendix-I. Papers I and II were published in Osteoporosis International in 2021 and the Journal of Bone Mineral Research in 2022, respectively. Paper-III is currently in the peer-review process.

3 Materials and methods

This thesis is based on the analyses of data from the longitudinal *AGES - Reykjavik study* (2002 to 2006), which forms the basis for the analyses performed in papers I to III. Below is a short description of the cohort and its main sources of data used for analyses.

3.1 Study population

The Icelandic Heart Association's longitudinal AGES - Reykjavik Study was conducted in collaboration with the National Institutes in the US, funded by their Intramural Research Program. The multidisciplinary study aimed to explore healthy aging and the environmental factors, genetic predispositions, and physical conditions that affected the health of elderly adults. Its results shed light on detailed phenotypes related to the musculoskeletal system, including information on cardiovascular and neurocognitive conditions. The AGES-Reykjavik study is a follow-up study of the Icelandic Heart Association Reykjavik Study, which surveyed a total of 30,795 participants who were born 1907 to 1935 and were living in the Capital Area in and around Reykjavik when recruited in 1967 to 1991. The original Reykjavik study sought to examine the distribution of cardiovascular disease in Iceland and determine the main contributing risk factors that could be targeted for preventative measures.

A total of 8030 participants from the original Reykjavik study were invited to participate in the AGES-Reykjavik Study between 2002 and 2006. A total of 5764 subjects aged 66 to 96 years old agreed to participate (72% of those invited), with all participants providing informed and written consent. The Icelandic National Bioethics Committee (VSN: 00-063) and the National Institute on Aging Intramural Institutional Review Board (MedStar IRB for the Intramural Research Program, Baltimore, MD) approved the study. The recruitment process and characteristics of participants have been described in detail elsewhere [129, 130].

3.2 Outcomes measures

A comprehensive data collection was conducted by way of participant enrollment (baseline) through detailed interviews, questionnaires, and clinical examinations. The main sources of information collected at baseline are summarized in this section.

3.2.1 Bone mineral density and bone health

During a clinical examination conducted at baseline volumetric bone mineral density (vBMD), bone mineral content (BMC) and bone volume of the femoral neck and trochanter were quantified using a Quantitative Computerized Tomography (QCT). Scans encompassing the proximal femur were obtained from a level of 1 cm above the acetabulum to a level 5 mm inferior to the lesser trochanter with 1 mm slice thickness. More details of the analytical procedures and quality assessment have been described elsewhere in a study by Johannesdottir and colleagues [131].

A total of 933 of the 5,764 participants recruited into the AGES-Reykjavik were either excluded from or were not able to undergo the QCT-analyses. The reason for participant dropout included inability to attend the clinical examination, exclusion from scanning due to weight (>150 kg), or having undergone a previous hip replacement procedure.

Clinical examination of participants' information on previous fractures was assessed using a self-reported questionnaire during the baseline. Participants were also asked if they had previously been diagnosed with osteoporosis.

3.2.2 Clinical biomarkers

Participants provided fasting blood samples as part of the baseline clinical examination, which was then separated, with the serum being kept frozen at -80˚C at the Icelandic Heart Association (IHA) laboratory. Based on these samples, several clinical biomarkers reflecting baseline health and nutritional status were quantified:

Vitamin D status: Serum 25(OH)D (D2 and D3) was quantified by chemiluminescence (CLIA) using the LIAISON 25-OH Vitamin D Total assay (DiaSorin, Inc., Stillwater, Minnesota). The coefficient of variation was <7% based on a pool of frozen serum samples from AGES-Reykjavik and <13% when using the Liaison quality control standard [97]. The serum 25(OH)D concentrations as quantified by the LIASION assay were then re-calculated based on the procedures developed by the NIH-led Vitamin D Standardization Program (VDSP) [132]. This scaling, used in our analyses, was based on reanalyzing serum 25(OH)D in a subset of samples using a certified LC-MS/MS method for re-calibration purposes from the National Institute of Standards and Technology's higher-order Reference Measurement Procedure.

Other clinical biomarker measurements measured at baseline included serum albumin, hemoglobin, and parathyroid hormone (PTH), for which standard determination methods were used. However, the measures of PTH were only performed in a subset of participants (34%).

3.2.3 Dietary intake

Participants' previous and current dietary habits were assessed using a self-reported food frequency questionnaire (FFQ). The questionnaire covered the intake of all main food groups, including milk and dairy products. As dairy is traditionally an important source of protein and nutrients in the Nordic countries, current dietary guidelines recommend an intake equivalent to two servings per day [76]. The questionnaire also inquired as to surveyees' use of dietary supplements, such cod liver oil, the primary supplemental source of vitamin D in older adults. This FFQ used in the Reykjavik-AGES study has previously been validated in reference to the use of cod liver oil supplements and consumption of milk and milk products alike [94, 133].

3.2.4 Body composition

Participants were asked to wear light underwear when their body weight was measured during the first visit to baseline. Height was also measured with a calibrated stadiometer during the first examination. To calculate body mass index (BMI), weight in kg was divided by measured height in meters squared. Bioelectric Impedance Analysis (BIA) was also used to measure fat and fat-free mass. The cross-sectional muscular area of the thigh and the subcutaneous fat located there was assessed using computed tomography (CT) [134, 135].

3.2.5 Measures of physical function

Several measures of strength and physical function were quantified at baseline which included:

Leg Strength: Muscle strength of the dominant leg was assessed as an individual's maximal isometric strength while sitting in an adjustable dynamometer chair (Good Strength, Metitur Ltd., Palokka, Finland). Knee extension (usually of the right knee) was measured in Newtons with the knee angle at 60° and the ankle fastened by a belt to a strain-gauge system. The best performance out of three attempts was recorded into the data file. This measure has been described previously [136].

Grip strength was quantified using a dynamometer, measured in Newtons, with the arm fixed and the elbow flexed at 90°. Participants were

given three attempts, with the best performance out of the three subsequently recorded. This has also been described in detail in another publication from the AGES study [137].

Timed up and Go (TUG) test: The measurement of TUG was one of several tests of participants' physical function. TUG measures the time it takes to stand up from a chair without support from the arms and then walk three meters, turn around, and sit down again. The time (in seconds) was measured using a stopwatch, and the better performance of two attempts was recorded [138, 139].

Gait speed was recorded by measuring the time it took to walk six meters both as fast as participants could and at their normal speed, measured by a stopwatch and using the best of two attempts [140].

Participants additionally underwent a **balance test**. The test was performed with participants standing with their feet separated from one other. The participants were asked to stand in a relaxed upright position on a force [postural] platform in the exact position of the V-feet placement with their hands relaxed [141]. Participants were then asked to look at a computer monitor. On the screen were five boxes, one in the center and one in each corner of the screen. Participants were asked to lean toward the box directions without losing balance or taking an extra step from the standing position. The maximal distance achieved was then recorded as a measure of balance in centimeters.

3.2.6 Other characteristics

Apart from socio-demographic variables including age, sex, education, and marital or cohabitant status, detailed information on participants' pasts and health backgrounds, including use of medication, was collected through a questionnaire that participants could request assistance in completing if needed.

Charlson's Comorbidity Index was assessed based on the information collected on participants' health. This index is a binary sum of the following comorbidities: Myocardial infarction, congestive heart failure, peripheral vascular disease, chronic obstructive pulmonary disease, diabetes and chronic renal failure. It is commonly used for clinical prognosis and comorbidity adjustment in related studies [142].

3.3 Assessment of hip incident fractures

Information was collected on incidence of hip fractures occurring from the

date of participants' recruitment until December 31st, 2012, which was extracted from hospital records based on International Classification of Diseases (ICD-10) codes S72.0, S72.1 and S72.2. An independent radiologist analyzed and verified all diagnoses in the records that were collected. It has been estimated that ~ 97% of hip fractures that occurred among participants were captured using this procedure [143]. Among the participants, the mean time (± standard deviation) from enrollment to the event among hip fracture cases was 7.4 ± 2.4 years.

3.4 Statistical analysis

Statistical analysis was performed by using SPSS for Windows version 22.0 or higher (IBM Corp. Armonk, NY, USA).

The mean and standard deviation (SD) were used to describe normally distributed continuous variables, while medians and percentiles were used to describe the skewed continuous variable. When testing for differences between two or more groups, the T-test or F-test were used for normally distributed continuous variables and the Mann-Whitney or Kruskal Wallis test was used for skewed variables. The distribution of all continuous variables was assessed using histograms and quantile-quantile plots. Dichotomous variables were described with percentages, and chi-squared tests were used to compare proportions across groups. The significance level was set for all statistical comparison at $p < 0.05$ (two-sided).

In covariate-adjusted analyses, multivariable linear regression and binary logistic regression were used to examine associations between participant's lifestyle or health characteristics with continuous or binary outcome measures, respectively. Cox regression was used to examine associations between participants' lifestyles or health characteristics and incidence of hip fractures. The underlying time scale used in the Cox regression models was the time from recruitment (started in September 2002 and ended in February 2006) until fracture, death or end of follow-up on the 31st of December, 2012. For papers-II and III, several covariates were included in fully adjusted models. Therefore, missing covariate values were imputed using multiple imputations (n = 5) as implemented in the missing value module in SPSS. In Paper-I, a complete case analysis was performed for each of the characteristics examined in that paper. The reason being that in that paper, several characteristics were explored in relation to hip fractures and adjustments were only performed for age and vBMD of femoral neck for which proportion of missing was low.

4 Results

The main results from papers-I to III are summarized in this section. Before presenting the main results, it is relevant to draw the reader's attention to the fact that the number of participants in each paper varied slightly. This is due to slight differences in inclusion criteria and missing values for exposure and outcome variables. The attrition for each paper from the total number of 5746 participants recruited into the Reykjavik-Ages study is shown in figure 4.

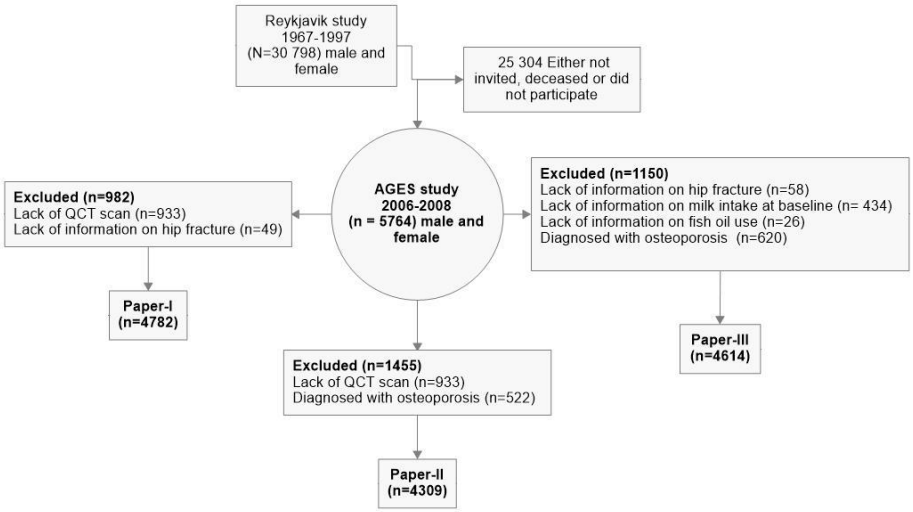

Figure 4: Flowchart showing the cohort attrition in papers-I to III from the total number of 5764 participants that were enrolled into the Reykjavik-AGES study.

The fewer number of participants included in paper-II than in paper-I (4309 vs. 4782) is primarily due to the decision to exclude participants diagnosed with osteoporosis in paper-II. The reason for this exclusion was the expectation that those who had been diagnosed with osteoporosis would have been advised to take vitamin D supplementation. Similarly, for paper-III, subjects diagnosed with osteoporosis were also excluded as it was suspected that those subjects might have been encouraged, based on medical advice, to increase their consumption of calcium (through dairy or

calcium supplements). The focus of paper-I was originally considered less dependent on such factors that might lead to reverse causation. As a result, these subjects who had been diagnosed were not excluded for the analyses in paper-I.

In order to characterize the potential differences in the included subjects in the three papers, a comparison was made for the main characteristics of the subjects, including: age, BMI, vBMD of femoral neck, serum 25(OH)D status and incidence of hip fractures cases. Those results are shown in Table 1. In brief, no major differences were observed in participants' mean age and baseline serum vitamin D status. No major difference was observed either for vBMD of femoral neck across the three papers for males. However, females in papers-II and III, where those previously diagnosed with osteoporosis had been excluded, had higher vBMD of femoral neck compared to the females included in paper-I (where all subjects were included).

Table 1. Main outcome variables for papers in different sub-samples. Using mean and standard deviation (SD).

	Male		**Female**	
	N	Mean (SD)	N	Mean (SD)
Age (years)				
Paper-I	2104	76.5 (5.4)	2678	76.2 (5.5)
Paper-II	2053	76.5 (5.4)	2256	76.0 (5.6)
Paper-III	2175	76.6 (5.4)	2439	76.3 (5.7)
vBMD (mg/cm^3)				
Paper-I	2104	254 (51)	2678	244 (50)
Paper-II	2053	255 (50)	2256	250 (49)
Paper-III	2175	255 (50)	2439	251 (50)
25(OH)D (nmol/L)				
Paper-I	2104	59.7 (17.9)	2678	55.3 (17.6)
Paper-II	2053	59.6 (17.9)	2256	54.7 (17.5)
Paper-III	2175	59.7 (18.0)	2439	54.8 (17.7)
Hip-fracture[1]		**n (incidence)**		
Paper-I	2104	114 (6.6)	2678	273 (10.8)
Paper-II	2053	114 (6.6)	2256	273 (10.8)
Paper-III	2175	117 (6.8)	2439	225 (8.9)

[1] Incidence rate per 1000 person-years.

4.4 Characteristics of participants that later had a hip fracture (Paper-I)

Table 2 shows the baseline characteristics of study participants in paper-I according to their later hip fracture status during the follow-up period (mean of 7.4 years (SD:2.4). Of the 2014 males included in this study, a total of 114 males experienced hip fractures (or 5.7%) during the follow-up period. In contrast, the rate was almost double, or 11.4% (273 of 24059), among females. On average, those experiencing hip fractures were ~3 years older than the non-fracture group. This age difference partly explains why mean values for most of the characteristics presented in table 2 are less favorable for hip fracture cases compared to non-cases. However, after adjusting for age (level of significance indicated by superscript in the table), many of the differences remained. For both sexes, those who later experienced hip fractures had, as expected, significantly lower baseline in vBMD of the femoral neck and both in the trabecular and cortical part compared to non-cases after adjustment for age. Serum 25(OH)D and albumin were also significantly lower among hip fracture cases in both sexes after adjustment for age. The muscle area of the thigh was also significantly lower in both sexes. In terms of sex-specific differences, female hip fracture cases had significantly lower BMI and fat-free mass compared to non-cases, but these differences were not observed in males after adjustment for age. In contrast, male hip fracture cases scored lower, after adjustment for age, in most of the measures of physical function such as balance, gait speed and TUG. These differences were not observed in females.

Table 2. Baseline characteristics of participants (N=4782).

	Males		Females	
	No fracture (n=1990)	Hip fracture (n=114)	No fracture (n=2405)	Hip fracture (n=273)
	Mean (standard deviation)			
General characteristics				
Age (years)	76.4 (5.3)	79.8 (5.5)[2]	75.8 (5.5)	79.2 (5.2)[2]
Bone mineral density of the femoral neck				
Integral BMD (mg/cm^3)	256 (50)	208 (41)[2]	248 (50)	215 (40)[2]
Trabecular BMD (mg/cm^3)	41 (42)	10 (34)[2]	25 (44)	6 (36)[2]
Cortical BMD (mg/cm^3)	544 (44)	529 (47)[2]	534 (42)	523 (40)[2]
Body composition				
BMI (kg/m^2)	26.8 (3.8)	26.0 (4.1)	27.4 (4.8)	25.6 (4.7)[2]
Fat free mass (kg)	64.0 (7.5)	61.6 (8.4)	46.0 (6.4)	43.4 (6.4)[2]
Fat mass (kg)	18.6 (7.0)	17.2 (7.0)	24.6 (7.4)	21.8 (7.4)[2]
Height (cm)	175.5 (6.2)	174.4 (6.7)	161.0 (5.7)	159.8 (5.7)
Muscle thigh area (cm2)	119 (21)	107 (20)[3]	85 (14)	77 (13)[2]
Biomarkers				
25(OH)D (nmol/L)[1]	57 (24)	52 (25)[3]	51(23)[2]	46 (22)
Hemoglobin (g/L)	14.1 (1.2)	13.7 (1.4)	13.2 (1.0)[3]	12.9 (1.1)
Albumin (g/L)	41.2 (2.6)	40.0 (3.0)[3]	40.9 (2.4)	40.5 (2.4)
Physical measurements				
Leg strength (N)	408 (108)	352 (184)[3]	262 (76)[2]	228 (67)
Grip strength (N)	389 (92)	363 (183)	236 (68)	217 (53)
Timed up and go (sec)	12.1 (3.2)	13.8 (3.7)[2]	12.4 (3.8)	13.3 (4.0)
Balance forw-backw (cm)	9.1 (3.0)	7.4 (3.2)[2]	7.9 (2.9)	7.1 (3.0)
Balance left –right (cm)	11.9 (3.2)	10.6 (3.7)[3]	9.7 (3.3)	8.9 (3.0)

[1]P value from independent samples' T-test and chi-squared test. Except for age all p-values are adjusted for age as a continuous measure; [2]25(OH)D = 25-hydroxy vitamin D; [3]P value <0.001; [4]P value <0.05.

Table 3 shows the relative baseline difference in measures of body composition, clinical biomarkers and physical function between those who later experienced hip fractures compared to those who did not. These relative differences are adjusted for both age and vBMD of the femoral neck and may as such reflect differences that are independent of those two factors.

For males, the most pronounced differences between hip fracture cases and non-cases were observed for physical function, with those experiencing later hip fractures having significantly worse performance in the Timed Up and Go test and balance test. Although formal significance was not reached, the leg strength among male hip fracture cases was also considerably poorer. In contrast, the main differences that characterized the females were lower BMI, fat and fat free mass and muscle thigh area among later hip fracture cases compared to non-cases. These differences were not seen in males. However, in both males and females, those who later experienced hip fractures had significantly lower serum 25(OH)D compared to non-cases with mean adjusted difference of around 5 nmol/L.

The association between the baseline characteristics presented in Table 3 are shown in Table 4 using Cox-regression analyses and calculate the hazard ratios (HR) with 95% confidence intervals (95%CI) for hip fracture risk. The hazard ratios reflecting change in risk per 1-SD increase in independent variables. The results presented are also adjusted for age and vBMD of femoral neck. As for table 3, a similar pattern is observed with associations showing lower risk (hazard ratio (HR)<1) for increased leg-strength and better performance in balance test and higher risk (HR>1) with worse performance in the Timed up and go test among males. In contrast, higher muscle thigh area, BMI and fat mass were associated with lower risk among females. Higher vitamin D status showed a near identical protective association for incident hip fractures in males [HR: 0.82 (95%CI 0.67, 1.00), p=0.06] and females [HR: 0.82 (95%CI 0.72, 0.94), p=0.002], although formal significance was only reached for females

Although the results in tables 3 and 4 largely reached the same conclusion, the two analyses reflect slightly different measures. Table 3 shows how measures of body composition, clinical biomarkers and physical function differ between later hip fracture cases and non-cases several years (on average) before hip fractures occurred, while the results in table 4 shows a measure of risk for an event, taking time of event into consideration. By the time the event (hip fracture) occurs, one would expect that these baseline measures may have changed considerably, and differently among cases and

non-cases. The results in table 3 and 4 strongly suggest that body composition, vitamin D status and physical function may influence risk of hip fracture independent of vBMD of femoral neck to a varying degree between the two sexes. This is important as both physical function, body composition and vitamin D status are to some extent modifiable factors that can be improved for prevention.

To follow up on these findings further, the dual role of vitamin D as a preventive factor for hip fracture, possibly mediated through bone health and physical function, was further explored.

Table 3. Differences in body composition, physical function and general health status between fracture vs non-fracture participants (N=4782).

	Adjusted for age and BMD[1]	*Adjusted for age and BMD[1]*
	Δ (95%CI)	*Δ (95%CI)*
Body composition		
BMI (kg/m^2)	0.1 (-0.7, 0.7)	**-2.0 (-1.6, -0.4)**
Fat free mass (kg)	-0.1 (-1.7, 1.5)	**-0.9 (-1.8, -0.1)**
Fat mass (kg)	0.1 (-1. 5, 1.6)	**-1.2 (-2.3, -0.2)**
Height (cm)	0.0 (-1.1, 1.2)	0.1 (-0.6, 0.8)
Muscle thigh area (cm^2)	-2.3 (-6.8, 2.3)	**-3.2 (-5.3, -1.14)**
Biomarkers		
25(OH)D (nmol/L)[2]	**-4.6 (-9.3, 0.0)**	**-4.6 (-7.5, -1.6)**
Hemoglobin (g/L)	-0.2 (-0.4, 0.1)	**-0.1 (-0.3, -0.0)**
Albumin (g/L)	**-0.8 (-1.5, -0.1)**	-0.2 (-0.6, 0.3)
Physical measurements		
Leg strength (N)	-19 (-41, 2)	**-14 (-23, -4)**
Grip strength (N)	2 (-17, 21)	-5 (-13, 4)
Timed up and go (sec)	**1.0 (0.4, 1.6)**	0.1 (-0.3, 0.6)
Balance forward-backward (cm)	**-1.0 (-1.6, -0.4)**	-0.1(-0.5, 0.3)
Balance left –right (cm)	-0.6 (-1.2, 0.1)	-0.2 (-0.6,0.2)

[1]BMD: integral bone mass density in the femoral neck region. [2]25OHD = 25-hydroxy vitamin D. Statistically significant estimates shown in bold folders

Table 4 Associations between baseline characteristics and risk of hip fracture over the follow-up period (N=4782

	Adjusted for age and BMD[2]	*Adjusted for age and BMD*[2]
	HR (95%CI) [1]	*HR (95%CI)* [1]
Body composition		
BMI (kg/m^2)	1.08 (0.88, 1.32)	**0.80 (0.69, 0.92)**
Fat free mass (kg)	1.10 (0.87, 1.39)	**0.87 (0.76, 1.00)**
Fat mass (kg)	1.11 (0.89, 1.39)	**0.85 (0.73, 0.99)**
Height (cm)	1.12 (0.93, 1.35)	1.02 (0.90, 1.17)
Muscle thigh area (cm^2)	0.78 (0.59, 1.03)	**0.66 (0.55, 0.81)**
Biomarkers		
25(OH)D (nmol/L) [3]	0.82 (0.67, 1.00)	**0.82 (0.72, 0.94)**
Hemoglobin (g/L)	**0.78 (0.63, 0.95)**	**0.84 (0.74, 0.95)**
Albumin (g/L)	**0.68 (0.51, 0.90)**	0.88 (0.73, 1.06)
Physical measurements		
Leg strength (N)	**0.70 (0.53, 0.92)**	**0.76 (0.65, 0.89)**
Grip strength (N)	0.89 (0.70, 1.22)	**0.73 (0.62, 0.85)**
Timed up and go (sec)	**1.53 (1.31, 1.79)**	**1.25 (1.12, 1.40)**
Balance forward-backward (cm)	**0.72 (0.60, 0.87)**	0.92 (0.81, 1.04)
Balance left –right (cm)	0.89 (0.74, 1.08)	0.91 (0.79,1.03)

[1]vBMD: Volumetric integral bone mass density in the femoral neck region. Statistically significant estimates are shown in bold font. [2]Hazard ratios (HR) and 95% confidence intervals (CI). The hazard ratios reflecting change in risk 1-SD increase in exposure. [3]25(OH)D = 25-hydroxy vitamin.

4.5 Vitamin D 25(OH)D hip fracture risk (Paper-II)

The aim of the second paper was to explore to what extent measures of bone mass and markers of physical function might, when considered jointly, explain the inverse associations between serum 25(OH)D and incidence hip fracture which was observed in paper-I.

When evaluating this association, Vitamin D status was divided into three categories as defined by the Institute of Medicine on the basIs of the effect of vitamin D on skeletal health [144].

- *Deficiency:* serum 25(OH)D < 30 nmol/L,
- *Inadequacy:* serum 25(OH)D 30 to <50 nmol/L
- *Sufficiency:* serum 25(OH)D ≥50 nmol/L.

In this study participants diagnosed with osteoporosis at baseline were excluded from the analyses (n = 522) as it was suspected that those subjects

would have been advised to take vitamin D supplement to improve their bone health. In such cases, it might be expected that their vitamin D status would improve well before any improvements in bone health would occur. Including those subjects might therefore lead to reverse causation.

The unadjusted cross-sectional association between vitamin D status and volumetric bone mineral density, bone volume and content of the femoral neck, is shown in Table 5. Higher serum 25(OH)D concentrations for both sexes were significantly associated with both vBMD and bone mineral content of the femoral neck; but not with bone volume. In these analyses, it is worth noting that in the case of males, vitamin D status was not associated with participant age (p=0.47). For females, however, those with deficient Vitamin D status (<30nmol/L) were approximately one year older compared to those with insufficient (30 to <50 nmol/L) and sufficient (≥50 nmol/L) status.

Table 5: Cross-sectional association between serum 25(OH)D at baseline and volumetric bone mineral density, volume and content of the femoral neck in participants of the Reykjavik AGES study (2053 males and 2256 females). Participants that had previously been diagnosed with osteoporosis were excluded from these analyses (n=522)

Males (n=2053)	<30 nmol/L (n=125)	30 - <50 nmol/L (n=465)	≥50 nmol/L[1] (n=1463)	P-Value[2]
	Mean (standard deviation)			
Age in years	77.0 (6.1)	76.3 (5.5)	76.6 (5.3)	0.47
BMI (kg/m^2)	27.25 (4.6)	27.19 (3.9)	26.69 (3.6)	0.02
Integral volumetric BMD (mg/cm^3)	240 (50)	251 (49)	257 (50)	<0.001
Bone mineral content (g)	4.91 (1.3)	4.99 (1.9)	5.1 (1.2)	0.01
Bone volume (cm^3)	20.52 (3.8)	20.08 (3.8)	20.25 (3.9)	0.50
Females (n=2256)	<30 nmol/L (n=236)	30 – <50 nmol/L (n=623)	≥50 nmol/L[1] (n=1397)	P-Value[2]
	Mean (standard deviation)			
Age in years	76.9 (6.0)	75.8 (5.2)	76.0 (5.7)	0.04
BMI (kg/m^2)	28.7 (5.8)	28.3 (5.1)	27.0 (4.4)	<0.001
Integral volumetric BMD (mg/cm^3)	242 (47)	251 (53)	252 (48)	0.02
Bone mineral content (g)	3.49 (0.8)	3.66 (0.9)	3.70 (0.8)	0.003
Bone volume (cm^3)	14.5 (2.7)	14.7 (2.8)	14.8 (2.7)	0.31

Abbreviation: vBMD, bone mineral density of the femoral neck. QCT, Quantitative computed tomography. [1]The median serum 25(OH)D concentration in each category was 25, 41 and 66 nmol/L, respectively. [2]F-test for continuous variables and chi-square test for dichotomous variables.

Having established that baseline vitamin D status was significantly associated with vBMD and bone mineral content of femoral neck in males and females in cross-sectional analyses, the next step was to examine how and to what extent vitamin D status is associated with incident hip fractures in this population. After adjustment for age and BMI (model 1 in Table 5), vitamin D status at baseline was significantly and inversely associated with incident hip fractures in both males and females, with a stronger effect size observed in males [HR 3.1; (95%CI, 1.9–5.2)] compared to females [HR 1.8; (95% CI, 1.3–2.5)] when comparing those with deficient (<30 nmol/L) versus sufficient ≥50 nmol/L. Further adjustment for vBMD of the femoral neck (model 2) reduced the HR for males from 3.1 down to 1.98 (95% 1.12, 3.59); while the effect estimate was essentially unchanged for females [HR: 1.82 (95%: 1.28, 2.59)]. When adjusting age BMI and markers of physical function, including leg strength, balance, and Timed Up and Go test, but not vBMD

(model 3), there were some reductions in the effect estimates compared to model 1 for both males (HR went from 3.1 to 2.5) and females (HR went from 1.82 to 1.63). However, in a fully adjusted analysis (model 4) where the marker of physical function and vBMD of the femoral neck were included jointly, the association became non-significant for males [HR: 1.26 (95%CI: 0.62, 2.54)] when comparing those with deficient versus sufficient status. However, the association was modestly reduced for females and was still significant [HR: 1.65 (95%CI: 1,12, 2.44)] compared to model 1.

Table 6. Associations between baseline serum 25(0H)D concentrations and incidence of hip fractures[1]

Serum25(OH)D	***Males***[1]		***Females***[2]	
(nmol/L)	*HR (95% CI)*	*P-value*	*HR (95% CI)*	*P-value*
Model 1[4]				
<30	3.08 (1.85, 5.15)	<0.001	1.80 (1.29, 2.50)	0.001
30 – <50	1.28 (0.85, 1.93)	0.24	1.14 (0.88, 1.48)	0.32
≥50	1.00		1.00	
P for trend[3]	0.002		<0.001	
Model 2[5]				
<30	1.98 (1.12, 3.50)	0.02	1.82 (1.28, 2.59)	0.001
30 – <50	1.21 (0.78, 1.89)	0.40	1.26 (0.97, 1.67)	0.08
≥50	1.00		1.00	
P for trend	0.04		<0.001	
Model 3[6]				
<30	2.53 (1.39, 4.62)	0.002	1.63 (1.12, 2.37)	0.01
30 – <50	1.11 (0.69, 1.77)	0.68	1.22 (0.92, 1.62)	0.17
≥50	1.00		1.00	
P for trend	0.03		<0.001	
Model 4[7]				
<30	1.26 (0.62, 2.54)	0.52	1.65 (1.12, 2.44)	0.01
30 – <50	1.17 (0.72, 1.91)	0.54	1.31 (0.98, 1.76)	0.07
≥50	1.00		1.00	
P for trend	0.20		0.004	

Abbreviations: HR, Hazard ratio. CI, confidence interval. Chi-square test [1]Males No of cases/N : <30 nmol/L 15/125. 30 – <50 nmol/L 28/465. ≥50 nmol/L 66/1463. [2]Females: No of cases/N : <30 nmol/L 35/236. 30 – <50 nmol/L 62/623. ≥50 nmol/L 113/1397 [3]P-value for lineral trend

[4]**Model 1**: Adjustment for age and BMI.
[5]**Model 2**: Model 1 with additional adjustment for volumetric bone mineral density of the femoral neck
[6]**Model 3**: Model 1 with additional adjustment for leg strength, balance and TUG.
[7]**Model 4**: All covariates in models 2 and 3.

As results in table 6 show, the association between vitamin D status and incident hip fractures for males was fully explained (or confounded) by the association between vitamin D status at baseline with vBMD of the femoral neck and markers of physical function for males. These factors could however not explain the association between vitamin D status and incident hip fractures for females. These stark differences are interesting given the fact that vitamin D status at baseline was, after adjustment for age, similarly associated with markers of physical function such as balance, leg- and grip strength and gait speed in males and females (Table 7). One the other hand, the association between vitamin D status and vBMD of the femoral neck, after adjustment for age, was stronger in males [mean difference: -17 mg/cm^3 (95%CI: -26, -8)] compared to females [-7 mg/cm^3 (95%CI: -14, -1)], when comparing those with deficient versus sufficient status. To put this effect size into perspective, the standard deviation for vBMD of the femoral neck for both sexes was ~50 mg/cm^3. The larger effect size observed for the cross-section association between vitamin D status and vBMD of femoral neck among males explains why adjustment for vBMD of the femoral neck when looking at association with incident hip fractures in table 6 was more influential

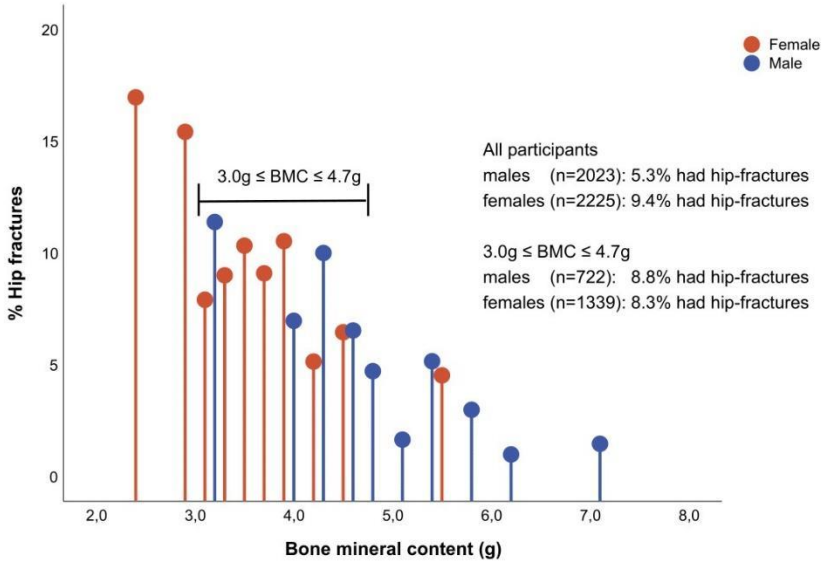

Figure 5. Incident hip fractures in relation to bone mineral content for males and females. The distribution was divided into deciles to plot the incident for fracture using median value in each decile. Where both males and females have substantial overlap (3.0 ≤ bone mineral content ≤ 4.7 g), similar prevalence of hip fractures (8%–9%) is

observed, whereas the lower overall prevalence among males (5.3%) compared to females (9.4%) appears to be driven by higher bone mineral content among males.

Although the strength of the association between vitamin D status and vBMD of the femoral neck at baseline differed slightly between males and females, this difference alone appears unlikely to account for the difference seen in table 6, where vBMD and measures of physical function fully explained the association observed for incident hip fractures in males but not females. One explanation for the sex-specific difference seen in table 6 might be due to the simple fact that, given the substantially lower overall bone mineral content, a measure of absolute bone strength, in males compared to females (as shown in table 5), the fracture risk in females is simply much higher in females, independent of vBMD of the femoral neck or physical function. This hypothesis is partly illustrated in figure 6, which plots the incident hip fractures among males and females according to their baseline bone mineral content of the femoral neck, which has been divided into deciles. The figure shows that in the interval (ca. 3.0 ≤ bone mineral content ≤ 4.7 g) where both sexes have more overlapping distribution, the incident is similar in both sexes (~8-9%). However, as females are overrepresented to the left of the graph, where the bone mineral content is low, the incident fractures are much higher (>15%) compared to the right side of the graph, where males are overrepresented (and the incident fractures are <5%). The lower bone mineral content of the femoral neck among females is largely explained by smaller bone mineral volume (see table 5), which is a non-modifiable determinant of bone mineral content.

In summary, in this study, population incident hip fractures appear similar between males and females when compared with subjects with similar bone mineral content. Higher fracture risk among females is largely explained by lower overall bone mineral content, which is partly driven by smaller bone volume. This may influence the differences observed for measures of physical function and vBMD of the femoral neck on incident hip fractures in males and females, and that is the underlying absolute bone mineral content may act as a modifying factor for these associations.

Table 7: Mean difference in baseline measures of body composition, volumetric bone mineral density and content and physical function according to baseline serum 25(OH)D status using the ≥50 nmol/L as a reference category with all comparisons age-adjusted.

	Serum 25(OH)D	Males N=2053	Females N=2256
	(nmol/L)	*Mean difference (95% Confidence interval)*[1]	
Body composition			
BMI (kg/m^2)	<30	0.6 (-0.1,1.3)	1.9 (1.2, 2.5)
	30 – <50	0.5 (0.1 0.9)	1.3 (0.9, 1.8)
	≥50	*Referent*	*Referent*
	P for trend[2]	*<0.001*	*<0.001*
Muscle area in the right thigh (cm^2)	<30	-6.1 (-10.5, -1.8)	-1.0 (-3.2, 1.3)
	30 – <50	-4.4 (-6.9, -1.9)	-0.9 (-2.5, 0.7)
	≥50	*Referent*	*Referent*
	P for trend	*0.008*	*0.68*
Subcutaneous fat in right thigh (cm^2)	<30	2.7 (-2.1, 7.5)	4.1 (-3.3, 11.5)
	30 – <50	6.1 (3.3, 8.8)	9.1 (3.8, 14.4)
	≥50	*Referent*	*Referent*
	P for trend	*<0.001*	*0.02*
Integral volumetric BMD (mg/cm^3)	<30	-17 (-26, -8)	-7 (-14, -1)
	30 – <50	-7 (-12, -2)	1 (-5, 4)
	≥50	*Referent*	*Referent*
	P for trend	*<0.001*	*0.003*
Bone mineral content (cm^3)	<30	-0.22 (-0.44, 0.01)	-0.17 (-0.28, -0.06)
	30 – <50	-0.16 (-0.29, -0.04)	-0.05 (-0.12, 0.03)
	≥50	*Referent*	*Referent*
	P for trend	*<0.001*	*<0.001*

Physical function			
Time up and go (sec)	<30	0.40 (-0.18, 0.97)	1.41 (0.93, 1.90)
	30 – <50	0.36 (0.04, 0.69)	0.53 (0.20, 0.86)
	≥50	*Referent*	*Referent*
	P for trend	*0.004*	*<0.001*
6m gait speed (sec)	<30	0.3 (0.1, 0.5)	0.4 (0.2, 0.5)
	30 – <50	0.2 (0.1, 0.3)	0.2 (0.1, 0.4)
	≥50	*Referent*	*Referent*
	P for trend	*<0.001*	*<0.001*
Leg strength (N)	<30	-15.9 (-35.1, 5.0)	-26.3 (-36.3,-16.3)
	30 – <50	-13.0 (-24.3,-1.7)	-9.0 (-15.8, -2.3)
	≥50	*Referent*	*Referent*
	P for trend	*0.13*	*<0.001*
Grip strength (N)	<30	-10.2 (-27.5, 7.2)	-7.8 (-17.1, 1.4)
	30 – <50	-11.5 (-21.4, 1.6)	-4.4 (-10.8, 1.9)
	≥50	*Referent*	*Referent*
	P for trend	*0.05*	*0.22*
Balance, backward – forward (cm)	<30	-0.37 (-0.92, 0.18)	-0.8 (-1.2, -0.4)
	30 – <50	-0.52 (-0.82, -0.21)	-0.3 (-0.5, 0.2)
	≥50	*Referent*	*Referent*
	P for trend	*<0.001*	*0.001*

Abbreviation: vBMD, volumetric bone mineral density of the femoral neck. [1]mean difference from the referent. 2Linear trend test result obtained by modeling serum 25(OH)D as a continuous variable in the regression model.

4.6 Consumption of dairy and incident hip fractures (Paper-III)

In the third and final paper of this thesis, the aim was to explore the association between milk consumption and cultured milk products and incident hip fractures in the AGES-Reykjavik study. A previous cross-sectional study on the association between milk and cultured milk products (hereafter referred to as “milk”) had reported slightly higher vBMD of the femoral neck among those drinking milk daily versus less than once a week [133]. However, associations with incident hip fractures were not examined in that study. Furthermore, no analyses had been performed examining the association between milk intake in the AGES participants with baseline measures of physical function and body composition. Exploring these associations seems relevant in this thesis, as milk is an important source of protein, calcium and phosphate in elderly subjects, which may be beneficial for musculoskeletal health.

The crude association between milk intake at baseline with body composition, measures of physical function and BMD are shown in Table 7 for both sexes combined. A total of 14% of participants (651/4614) consumed less than 0.5 servings (~250 mL) per day, while 22% of participants consumed two servings or more, as recommended in national dietary recommendations [76]. Higher milk intake at baseline was relatively strongly associated with higher vBMD of the femoral neck and vitamin D status. The association with vitamin D status is most likely related to a higher intake of fish oil among high milk consumers (also shown in Table 8).

Table 8. Estimated means of variables associated with bone health categorized by milk consumption among AGES-Reykjavik participants as recorded at recruitment into the study (n=4614).

Variable	< 0.50 servings/day n= 651	0.50 - 1.99 servings/day n=2934	≥2 servings/day n=1029	P for trend*
	Mean (95% Confidence interval)			
BMI (kg/m2)	27.3 (26.9 - 27.6)	27.3 (27.1 - 27.4)	27.1 (26.8 - 27.4)	0.583
Fat free mass (kg)	54.6 (54.0 - 55.2)	55.2 (54.9 - 55.4)	55.5 (55.0 – 56.0)	0.076
Bone mineral density (mg/cm3)	248 (244 – 252)	252 (250 – 254)	258 (255 – 261)	<0.001
TUG (sec)	12.5 (12.3 – 12.8)	12.4 (12.3 – 12.5)	12.6 (12.3 – 12.8)	0.402
6 m gait speed (sec)	6.7 (6.6 – 6.9)	6.6 (6.5 – 6.7)	6.7 (6.5 – 6.8)	0.375
Leg strength (N)	330 (323 – 337)	333 (330 – 337)	328 (322 – 334)	0.245
25(OH)D (nmol/L)	54.2 (52.8 – 55.6)	57.0 (56.4 – 57.7)	59.7 (58.7 – 60.8)	<0.001
Lean for-back (cm)	8.3 (8.1 – 8.6)	8.3 (8.3 – 8.5)	8.4 (8.2 – 8.6)	0.872
	OR (95% Confidence interval)			
Fish oil daily	0.42 (0.34 – 0.52)	0.68 (0.58 – 0.80)	1.00 REF	<0.001
Calcium supplements	0.95 (0.71 – 1.28)	0.88 (0.71 – 1.11)	1.00 REF	0.515

*P-values are age and sex-adjusted and estimated using a general linear model for continuous variables (F-test) and logistic regression for categorical variables (Chi-square test).

The association between milk intake at baseline and incident hip fractures are shown in Table 9 for both sexes combined. When adjusting for age and sex (model 1), those consuming less than 0.5 servings of milk per day had a 1.59 (95%CI: 1.10, 2.28) higher risk of incident hip fractures compared to those consuming ≥2 servings a day, as reflected by the hazard ratios. Further adjustments for education, marital status, smoking, alcohol, physical activity, number of medications, TUG, and balance (model 2) did not affect the risk estimates. However, after further adjustment for vitamin D status and vBMD of the femoral neck, the corresponding effect estimate was slightly reduced but still significant [HR: 1.46 (95%CI: 1.01, 2.11)].

When examining the sex-specific associations between milk intake and incident hip fractures were explored, the effect size was stronger in males [HR: 2.12 (95%CI: 1.05, 4.24)] compared to females [HR: 1.49 (95%CI: 0.91, 2.14)], when comparing those consuming <0.5 versus ≥2 servings per day (see supplemental table S1 in paper-III). These sex-specific differences were also reflected in slightly stronger cross-sectional association between milk intake and vBMD of the femoral neck in males compared to females (see supplemental table S1 in paper-III).

Table 9. Risk of hip fracture* of participants (N = 4614) categorized by milk consumption.

	milk servings/day	HR (95% Confidence interval)	P-value
Model 1	< 0.5[1]	**1.59 (1.10 – 2.28)**	0.013
	0.5 - 0.9[2]	**1.56 (1.09 – 2.25)**	0.016
	1.0 - 1.4[3]	1.31 (0.96 – 1.79)	0.087
	1.5 - 1.9[4]	1.21 (0.85 – 1.72)	0.291
	≥ 2[5]	1.00 (ref)	ref.
Model 2	< 0.5	**1.60 (1.11 – 2.30)**	0.012
	0.5 - 0.9	**1.56 (1.08 – 2.26)**	0.017
	1.0 - 1.4	1.34 (0.98 – 1.83)	0.069
	1.5 - 1.9	1.25 (0.87 – 1.77)	0.225
	≥ 2	1.00 (ref)	ref.
Model 3	< 0.5	**1.46 (1.01 – 2.11)**	0.045
	0.5 - 0.9	**1.48 (1.02 – 2.14)**	0.037
	1.0 - 1.4	1.30 (0.95 – 1.78)	0.102
	1.5 - 1.9	1.30 (0.91 – 1.86)	0.145
	≥ 2	1.00 (ref)	ref.

*Cox regression; P-value (Chi-square test) for linear trend (based on group medians) for model 1: p = 0.004, for model 2: p = 0.004 and for model 3: p= 0.025. [1]Participant/cases n=651/55,[2]Participant/cases n=622/54, [3]Participant/cases n=1438/109, [4]Participant/cases n=874/61, [5]Participant/cases n=1029/63.

Model 1: adjusted for age and sex;

Model 2: additionally adjusted for education, marital status, smoking, alcohol, physical activity, number of medications, TUG, balance;

Model 3: additionally adjusted for 25(OH)D and vBMD of the femoral neck.

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5 Discussion

A hip fracture can be a life-changing event for older adults, specifically regarding high mortality and morbidity [145, 146], as well as cause financial burden to society [147]. Identifying risk factors that may, through suitable intervention, reduce the risk of fractures is therefore of considerable public health importance. Ideally, this should be examined in a different setting as there are substantial differences in the prevalence of hip fractures across countries that may reflect cultural and lifestyle differences as well as health care settings [25]. As such, the AGES Reykjavik longitudinal study provides a unique opportunity to examine risk factors in older adults, reflecting lifestyle habits and behaviors in Northern latitudes where exposure to sunlight is scarce, and access to health care makes identification of hip fractures reliable and accurate.

5.4 Paper-I: Characteristics of incident hip fracture cases in the AGES Reykjavik Study

After adjustment for age and vBMD of the femoral neck, distinct sex-specific differences for those experiencing hip fractures compared to non-cases were observed in the AGES Reykjavik study. Male cases were primarily characterized by worse performance in tests reflecting physical function such as balance and TUG, while female cases were primarily characterized by lower BMI, fat- and fat-free mass. Some similarities were observed in both sexes, including lower vitamin D and albumin status, as well as poorer leg strength and muscle thigh area, although formal significance was not reached for both sexes for all these measures.

Our findings on lower BMI, fat- and fat-free mass as a predictor for hip fractures in females is consistent with conclusions from systematic reviews and meta-analyses suggesting that lower BMI is associated with lower bone mineral density (BMD) and higher risk of fractures in females [148-150]. Similar findings have been reported for other measures of body composition, including absolute weight [68] in both sexes combined. In these meta-analyses, no adjustment for BMD was made. However, in analyses of 12 prospective studies including around 60,000 subjects, the inverse association between BMI and incident hip fractures became non-significant after adjustment for BMD. It is difficult to compare the results of our study directly

to this study as sex-specific associations were not reported, but our results suggested that the adjustment for vBMD of the femoral neck was more influential for males compared to females. Some controversy still exists about body composition, as one systematic review concluded that abdominal obesity was significantly associated with an increased risk of fractures [151]. However, in our study, associations with central obesity could not be addressed, hampering direct comparison.

Concerning our findings that poor performance of physical function was more pronounced in males than females, we are not aware of any studies making similar sex-specific comparisons directly. However, the results from the EPIDOS study, published in the Lancet in 1996, showed strong associations with measures of physical function, including physical disability score, tandem walk and gait speed in both sexes [121]. Like our study, these differences were still present after adjustment for BMD.

Our findings on the muscle thigh area are associated with around 34% and 22% lower risk of hip fractures per 1-SD increase, and similarly, our findings on vitamin D status, as reflected by serum 25(OH)D status, show a protective association is consistent with findings from the observational literature [152, 153]. Similarly, lower albumin status observed at baseline among incident hip fracture cases in both sexes is in line with studies associating anemia with a higher risk of fractures [154, 155].

It is possible that some of the sex-specific differences in our study may have occurred by chance. Furthermore, focusing on strict statistical significance when comparing associations between different risk factors can be problematic given the different incidents in males and females (thereby statistical power). The lack of studies exploring sex-specific differences limits the comparison of our studies with many previous reports. However, our study clearly suggests that there are indeed sex-specific differences when it comes to risk factors for hip fractures in older adults.

5.5 Paper-II: Serum 25(OH)D and its association with vBMD of the femoral neck and hip fractures

The primary focus of our analyses in this paper was to explore the relationship between vitamin D status at entry into the AGES Reykjavik study (baseline) in relation to vBMD of the femoral neck (cross-sectional analyses) as well as the risk of incident hip-fractures (prospective analyses). Furthermore, the secondary aim was to assess to what extent vitamin D status at baseline might be related to measures of physical function in males and females and to what extent these measures might explain any associations between vitamin D status with vBMD and fracture risk.

Participants who had been diagnosed with osteoporosis when entering the AGES Reykjavik study were excluded in our analyses to avoid possible reverse causation. That is, it was expected that participants who had been diagnosed with osteoporosis would have been advised to take vitamin D supplements which might lead to spurious correlation between vitamin D status, bone mass and later fractures.

Our results were in line with other observational studies showing a higher risk of hip fractures among those with low vitamin D status [105]. This has particularly been the case in the few studies examining associations with vitamin D deficiency, defined as serum 25(OH)D below 30 nmol/L [156], as done in our study. Similar to our cross-sectional findings, serum 25(OH)D levels have consistently been observed to be inversely associated with bone mineral density. Findings that are also supported by findings from randomized controlled trials [71].

Our analytical approach of adjusting for vBMD of the femoral neck and measure of physical function in two separate models, and then combined, was prompted by previous analyses on vitamin D and hip fractures in the AGES Reykjavik study [97]. Unlike our study, the previous analyses included those diagnosed with osteoporosis at baseline. In this previous study, the observed association with incident hip fractures was stronger than what would have been expected from the association observed between serum 25(OH)D and vBMD of the femoral neck alone [97]. Furthermore, growing evidence for possible effects of poor vitamin D status on physical function [157] provided clear justification for examining if such factors could influence any fracture risk associated with vitamin D status in our study. In particular, randomized controlled trials showing reduced propensity for falls following vitamin D supplementation [158] provide perhaps quite convincing evidence

that any effect of vitamin D status on risk of fractures may not be fully explained by effects on bone mass alone.

Our results partly confirmed our hypothesis as adjustment for measures reflecting physical function reduced the observed association between vitamin D status and fracture risk in males. Combined adjustment for measures of physical function and vBMD of the femoral neck completely eliminated the inverse relationship between serum 25(OH)D and hip fractures among males, confirming the substantial contribution of both factors. On the other hand, a similar influence was not observed for females, where adjustment for both factors (i.e. vBMD of the femoral neck and measures of physical function) did fully account for the inverse association observed between vitamin D status and hip fractures. This observation is somewhat surprising given the fact that vitamin D status was to a large extent similarly associated with measures of physical function in both males and females in our study (see table 7).

One possible explanation for the sex-specific difference observed in our study may be related to differences in bone mass between males and females (see table 5). Since females have on average smaller bone volume than males, the absolute bone mass (a direct measure of bone strength) is on average substantially lower in females compared to males. As such, slightly higher bone mineral density may not be sufficient to compensate for small bone size at an older age. This is clearly shown in figure 6 where incident fractures for males and females are shown across deciles of absolute bone mass of the femoral neck (vBMD x bone volume) for males and females. As expected, given the lower bone mineral content among females, their fracture risk is elevated, but in the interval where both males and females had similar bone mineral content, their fracture risk was similar. It appears to some extent reasonable that factors such as physical function may differently affect fracture risk among males and females when differences in bone mass, thereby fracture risk, are so different between the two sexes. This explanation has been noted by other researchers [159, 160], but it has only been minimally addressed.

5.6 Paper-III: Consumption of dairy and incident hip fractures. Relevance of existing recommendations

Milk contains minerals and vitamins, which are essential for good bone health. In Iceland, milk is widely consumed in older adults with typical intakes of ~300 - 400 g/day, providing 40% of their dietary calcium [82, 161, 162].

There is sufficient scientific evidence that milk and dairy consumption are inversely related to bone turnover and positively related to BMD [163, 164]. Despite this, the relationship between milk consumption and the risk of hip fracture is less clear and current epidemiological evidence indicates no clear overall association between milk intake and hip fracture risk [84, 165].

The Nordic Nutrition Recommendations 2012 suggest regular milk and dairy intake is equal to two servings per day as part of a healthy dietary pattern which ensures bioavailable calcium, which is important for long term bone health [76, 166]. Interestingly though, only around 23% of participants in the present study consumed at least two milk servings per day.

The results from our study suggest that regular milk intake may be positively associated with vBMD of the femoral neck (based on cross-sectional analyses) for both sexes. More importantly, we found that higher milk intake was associated with substantially lower risk of hip fracture in the longitudinal analysis.

The lowest milk category (< 0.5 servings/day) in our study had around a 59% higher risk of hip fracture when compared to the highest milk category (≥2 servings/day). When males and females were examined separately, the associations between milk intake and hip fracture risk were stronger in males than females, but the direction of the association was the same in both sexes. These observed associations were robust, and further statistical adjustment for confounding variables did only marginally change the hazard ratios, and the associations remained significant. In a more detailed dose-response analysis, in which we categorized participants according to milk intake into five groups (or when modeling milk intake as a continuous variable), the data also showed an inverse linear relationship between milk consumption and hip fracture risk.

In general, lowering the risk of hip fracture in older adults is important as the morbidity rate is high, and hip fracture is associated with low quality of life, dependence, and caregiver burden [167]. The mortality rate is usually high in hip fracture cases, and a recent Icelandic study showed that 36% of male and 21% of female patients died within a year from hip fracture [18].

5.7 Implications of Nursing Practice

Registered nurses are toned to be involved in preventing or delaying hip fractures for older adults. Their role is important in several areas of health care, such as primary health care, home care, emergency care, acute care, and rehabilitation. For example, the community-dwelling older adult meets

registered nurses in primary health care and home care, where the opportunity for preventive measures would be of most value. The findings of this study stress the value of a diet rich in vitamin D and calcium as well as an active lifestyle. Furthermore, they suggest the importance of screening older adults for serum 25(OH)D to find those at greater risk for hip fractures. When meeting patients, nurses have the opportunity to advise and educate their clients and promote a healthier lifestyle. This entails advising both males and females to exercise for balance and muscle strength, and for best results, take vitamin D supplements and calcium through dairy or supplements [102]. Also, resistance training for better muscle and bone strength and balance training should be encouraged. This may be of more importance for females than males.

In the emergency department, the nurse will meet older adults admitted after falling, who hopefully have escaped without breaking any bones. Screening this group is vital because of their increased risk of fractures. This contact with the elderly person provides the nurse with an opportunity for implementing the first preventive measures. It is essential to offer them information and guidance on lowering the risk of repeated falls and possible hip fractures and refer them for a follow-up appointment, such as at their primary health care. Research has shown decreasing fall rates with nurse-led fall-prevention services [168].

Preventive measures are also important for other older adults who have already had a fracture. Serum levels of vitamin D need to be measured, their use of vitamin D and calcium supplements need to be evaluated, and their overall nutrition status. Further rehabilitation following a fracture needs to include balance training, exercise, and physical therapy. Regarding the findings of this study, hemoglobin status needs to be measured before discharge. Preventing hip fractures and preventing second hip fractures is one of the primary roles of registered nurses in acute and orthopaedic services. Furthermore, providing hip fracture patients with expert nursing care has also decreased the length of stay and lower mortality [169].

5.8 Strengths and limitations

The strength of the three studies on incident hip-fractures presented in this thesis is the longitudinal study design, detailed clinical evaluation of participants at baseline, large sample size, and near complete (~97%) registry-based follow-up for incident hip fractures. Being able to include many important and relevant covariates in our adjusted analyses (i.e., biochemical, anthropometric, functional, socioeconomic, and health-related variables) is also an added strength. Furthermore, using a QCT- scan to quantify bone mass of the femoral neck (i.e., vBMD and BMC), giving a three-dimensional direct measure of volume provided, added a level of detail. Additionally, information about osteoporosis at baseline was also important as inclusion of those participants might be a source of reverse causation for our analyses on vitamin D.

In terms of limitations, the observational nature of our analyses is prone to bias, including confounding, making strong conclusions on causality difficult. Furthermore, several of our analyses, when examining associations between factors recorded at baseline, were cross sectional and such studies are especially prone to reverse causation. In addition, our analyses were based on single baseline measurements for several biomarkers, such as serum 25(OH)D, which is a limitation taking into consideration the known seasonal variation of vitamin D. Lack of information on previous physical activity and relying on imprecise measures of body composition though bioelectric impedance to assess fat and fat-free mass can also be mentioned as a limitation. Finally, the food frequency questionnaire used in our study only covered main food groups, and in the case of dairy, it did not contain any questions on cheese, which is an important source of calcium. One may also suspect that the accuracy of reporting on diet among these older adults might to some extent depend on their health status, which could confound the association with hip fractures.

In conclusion, despite many strengths, the interpretation of our observational findings needs to be considered in the context of other studies where sources of bias may have differed.

6 Conclusions

The aim of this thesis was to investigate characteristics of hip fracture cases compared to non-cases in AGES-Reykjavik Study from the Icelandic Heart Association. Hip fracture cases generally had lower muscle mass than non-fracture cases. Accordingly, hip fracture cases compared to non-cases generally performed worse in measures capturing physical strength and physical function, including TUG test, leg-strength and balance. This is an important finding because lower muscle mass and strength on a group level may indicate a higher prevalence of sarcopenia among those participants. More frequent screening among older adults for sarcopenia could perhaps lead to more targeted intervention that could lower associated comorbidities such as hip fractures. It is also important to stress that physical strength and function are modifiable factors that can be measured using simple, non-expensive tests (such as TUG) by healthcare professionals. Such screening, if applied routinely, could lead to early interventions and may have the potential to lower the burden of hip fractures on the health care system and improve the quality of life among older adults.

Another aim of this study was to investigate 25(OH)D in relation to hip fracture risk factors other than vBMD of the femoral neck. Low 25(OH)D was associated with several risk factors for hip fractures (e.g., low muscle mass, poor balance and low physical strength). Interestingly, the association between vitamin D status and incident hip fractures for males was fully explained by the association between vitamin D status at baseline with bone mineral density and markers of physical function for males. These factors could, however, not explain the association between vitamin D status and incident hip fractures for females. An explanation for this sex-specific difference might be due to lower overall bone mineral content in females compared to males, resulting in much higher fracture risk in females independent of vBMD of femoral neck or physical function. In terms of clinical practice, this is an important finding as it may suggest that the causes of hip fracturs may not be that sex-specific in terms of behaviour and lifestyle, suggesting that prevention should be similarly targeted for both sexes.

Finally, we also investigated the associations between milk intake and hip fracture risk. Milk intake as recommended (≥2 serving/d) was only achieved by around 23% of the participants. At that level, milk intake was associated

with significantly higher vBMD of the femoral neck at baseline and with a significantly lower risk of hip fracture during follow-up when compared to the lowest milk intake category. Further dose-response analyses showed an inverse relationship between milk intake and hip fracture risk. These findings suggest that simple compliance to official dietary recommendations on dairy consumption, as well as on maintaining sufficient vitamin D status through the use of supplements, may have significant benefits in terms of prevention.

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Original publications

Paper I

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ORIGINAL ARTICLE

Characteristics of incidence hip fracture cases in older adults participating in the longitudinal AGES-Reykjavik study

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Abstract

Summary Poor physical function and body composition my partly predict the risk of falls leading to fracture regardless of bone mineral density.

Introduction To examine the relationship between body composition, physical function, and other markers of health with hip fractures in older community-dwelling Icelandic adults.

Methods A prospective cohort of 4782 older adults from the AGES-Reykjavik study. Baseline recruitment took place between 2002 and 2006, and information on hip fractures occurring through 2012 was extracted from clinical records. Using multivariate regression analyses, baseline measures of bone health, physical function, and body composition were compared between those who later experienced hip fractures and to those who did not. Associations with the risk of fractures were quantified using Cox regression.

Results Mean age was 76.3 years at baseline. After adjustment for age, regression showed that male hip fracture cases compared with non-cases had (mean (95% confidence interval)) significantly lower thigh muscle cross-sectional area − 5.6 cm^2 (− 10.2, − 1.1), poorer leg strength − 28 N (− 49, − 7), and decreased physical function as measured by longer timed up and go test 1.1 s (0.5, 1.7). After adjustment for age, female cases had, compared with non-cases, lower body mass index − 1.5 kg/m2 (− 2.1, − 0.9), less lean mass − 1.6 kg (− 2.5, − 0.8), thigh muscle cross-sectional area − 4.4 cm^2 (− 6.5, − 2.3), and worse leg strength − 16 N (− 25, − 6). These differences largely persisted after further adjustment for bone mineral density (BMD), suggesting that body composition may contribute to the risk of fracture independent of bone health. When examining the association between these same factors and hip fractures using Cox regression, the same conclusions were reached.

Conclusions After accounting for age and BMD, older adults who later experienced a hip fracture had poorer baseline measures of physical function and/or body composition, which may at least partly contribute to the risk of falls leading to fracture.

Keywords Aging · Biomarkers · Body composition · Hip fracture · Physical function

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Introduction

Hip fractures constitute a major health problem for the elderly in terms of reduced quality of life and life expectancy [1]. The associated financial burden for society is also substantial with estimated health care costs per fracture ranging between $3500 and $25,000 [2]. At an older age, the increased incidence of hip fractures can largely be explained by the critical loss of bone mass combined with a deterioration of physical function resulting in an increased propensity for falls [3]. Although a number of risk factors for hip fractures have been identified, many of them such as age, height, and sex [4–6] are non-modifiable and of limited use for prevention. Modifiable risk factors that directly influence BMD include vitamin D and calcium [7–10]. Other factors such as smoking [11], low body weight [12], exercise [10, 13, 14], physical function [15, 16], and balance [17, 18] appear to have a more dual role as they may affect both BMD and propensity of falls.

Many previous studies have often focused on quantifying fracture risk for a single or a few selected risk factors only. This approach is somewhat limited, as it largely ignores the fact that risk factors tend to emerge in clusters at an older age when health deteriorates [19, 20]. In terms of prevention, a better understanding of how individual risk factors for hip fractures cluster and identifying through which pathway they may operate is likely to achieve better results than focusing on individual risk factors alone.

In a large prospective cohort of elderly subjects who underwent detailed clinical examination, we examined the baseline characteristics of individuals who subsequently had hip fractures (cases) compared with those who did not (non-cases) in relation to measures of bone health, body composition, physical function, lifestyle, and health. We aimed to identify and separate risk factors for hip fractures associated with an increased propensity for falls on the one hand and those associated with poor bone health on the other.

Methods

Study participants

This study is based on *Age, Gene/Environment Susceptibility-Reykjavik Study* (AGES-Reykjavik) that has been described in detail elsewhere [21]. Between 2002 and 2006, a total of 5764 subjects were recruited, 3326 female and 2438 male. A total of 933 subjects did not undergo QCT scanning either because they did not want to attend or because they excluded for reasons including extreme body weight, being unable to lie flat on the scanner bed, and having metal implants at the scan site. In addition, information on hip fracture status during follow-up was missing for 49 subjects leaving 4782 subjects (84% of those enrolled) available for analyses. The subjects not included in our analyses were on average 4 years older. After adjustment for age, those who were not included had poorer physical function as measured by the timed up and go test and leg strength. There were, however, no marked differences in clinical biomarkers (including serum 25(OH)D) and body composition (see Supplemental Table 1).

All participants provided written informed consent. The study was approved by the Icelandic National Bioethics Committee (VSN: 00-063) and the National Institute on Aging Intramural Institutional Review Board (MedStar IRB for the Intramural Research Program, Baltimore, MD).

Assessment of hip fractures

Hip fractures were defined according to the International Classification of Diseases version 10, diagnostic codes S72.0, S72.1, and S72.2 [22]. Based on this definition, information on hip fractures were extracted, verified, and confirmed by medical and radiological records. The exact procedures have been described previously, where it was estimated that around 97% of all hip fractures occurring among participants were captured [23]. Information on hip fractures included all events occurring from participants' enrollment (2002–2006) into the study until 31 December 2012.

Clinical examination

Blood chemistry

During the clinical examination, fasting blood samples were drawn, and biomarkers reflecting general health and nutrition were quantified, i.e., serum 25-hydroxy vitamin D (25(OH)D), albumin, and hemoglobin using standard methods [7].

Body composition

Body weight was measured in light underwear on a calibrated scale (model no. 708, Seca, Hamburg, Germany), and height was measured with a calibrated stadiometer (model no. 206; Seca, Hamburg, Germany). Based on these two measures, sex-specific fat- and fat-free mass were estimated using bioelectric impedance analysis (BIA). Body mass index (BMI) was calculated as weight in kg divided by height in meters squared.

Muscular strength, physical function, and balance

Leg strength and grip strength were quantified using a computerized dynamometer chair (Good Strength, Metitur Ltd., Finland). Leg strength was measured in the right knee and quantified in terms of the maximal isometric extension force, as described previously [24]. Grip strength was quantified in

terms of the maximum force the participants could squeeze as has been described before [25]. For both leg and grip strength, the best performance out of three measures was used. Physical function was assessed as the timed up and go test, which measures the time it takes to stand up from a chair, walk 3 m, return, and sit down [26, 27]. Participants also underwent a balance test [28], where they were asked to stand in an eased upright position with hands beside the body. The test consisted of following a computer monitor that showed a moving frame of reference that should be followed without losing balance or taking an extra step. The maximal distance achieved was then recorded for leaning backwards and forward and when leaning to the sides from left to right.

Assessment of bone health and muscle thigh area

Bone health was measured using quantitative computerized tomography (QCT) measuring bone mineral content, volume, and density of the femoral neck and trochanter. The integral bone mineral density was then calculated from those measures. The exact procedures and quality control for these scannings have been described in detail elsewhere [29].

Information on lifestyle and health

During the clinical examination, participants filled out a questionnaire covering medical history, lifestyle, and socioeconomic status. To summarize the health status of participants, we used the Charlson comorbidity index (CCI) [30] defined as the binary sum over the following comorbidities: myocardial infarction, congestive heart failure, peripheral vascular disease, chronic obstructive pulmonary disease, diabetes, and chronic renal failure as described by Austin et al. [31]. Current alcohol consumption was categorized as higher or lower than moderate intake of $\leq$ 25 g/day or > 25 g/day [32]. Current smoking was categorized as yes/no. Physical activity was categorized as $\leq$ 30 or > 30 min of moderate or vigorous physical activity per day. The number of falls during the last 12 months before AGES recruitment was categorized as $\leq$ 3 or > 3 falls.

Statistical analyses

Statistical analysis was conducted using SPSS for Windows version 24.0 (SPSS, Chicago, IL, USA), and the level of significance was set at $P < 0.05$. Data were checked for normality using visual inspection of histograms and quantile-quantile plots. The mean and standard deviation (SD) were used to describe continuous variables, and percentages were used to describe dichotomous variables.

Differences between hip fracture and non-fracture cases were examined using multivariable linear regression analyses, run separately for males and females. For these analyses, adjusted models accounting for either (1) age or (2) age and BMD of the femoral neck were used. Differences between hip fracture cases and non-cases were formally tested using the *t* test that was for continuous variables and chi-square test for dichotomous variables.

In addition to examining differences in characteristics of hip fracture cases and non-cases at baseline, Cox regression analysis was also used to quantify the association between baseline measures of body composition, clinical biomarkers, physical function, and later fracture status. As mentioned above, adjustments for either (1) age or (2) age and BMD of the femoral neck were made. The underlying time scale in these models was the time from recruitment (baseline) until fracture, death, or end of follow-up. The hazard ratio was estimated in relation to a 1-SD increase in the independent variable.

Results

Among 2104 males and 2678 females with BMD data, a total of 114 and 273 hip fractures occurred between recruitment (2002–2006) and end of follow-up (31 December 2012), respectively. The mean (SD) age at hip fracture was 79.8 years (5.5) for males and 79.2 years (5.2) for females with mean (SD) follow-up time of 4.6 years (2.3) and 5.3 (2.4) for males and females, respectively. The mean follow-up time for non-cases was 7.4 (2.4) and 7.9 (2.1) for males and females, respectively. No clear seasonal variation in hip fractures was observed (data not shown).

Crude measures of bone health, body composition, clinical biomarkers, physical function, and other characteristics recorded at baseline among those who later experienced hip fractures and those who did not are presented in Table 1. After accounting for age (age-adjusted *P* values), males and females who later experienced hip fractures had significantly lower baseline serum 25(OH)D and BMD of the femoral neck compared with non-cases. More pronounced differences in BMD were observed for the trabecular compared with the cortical part of the bone. For both sexes, hip fracture cases also had poorer leg strength and were more likely to experience several falls (> 3) during the previous 12 months prior to recruitment. No differences in Charlson comorbidity index and smoking were observed. With respect to sex-specific differences, male cases had significantly worse balance, lower albumin concentrations, and performed worse in the timed up and go test compared with non-cases. On the other hand, more pronounced and significant differences in body composition were observed between female cases and non-cases.

Adjusted differences between hip fracture cases and non-cases are shown in Table 2. Compared with the age-adjusted model, further adjustment for BMD had only a minor impact on the observed differences between hip fracture cases and

Table 1 Baseline characteristics of participants ($N = 4782$)

	No. Males/females	Males		Age-adjusted P value[1]	Females		Age-adjusted P value[1]
		No fracture ($n = 1990$)	Hip fracture ($n = 114$)		No fracture ($n = 2405$)	Hip fracture ($n = 273$)	
		Mean (standard deviation)			*Mean (standard deviation)*		
General characteristics							
Age (years)	2104/2678	76.4 (5.3)	79.8 (5.5)	< 0.001	75.8 (5.5)	79.2 (5.2)	< 0.001
Charlson comorbidity index	2104/2678	1.4 (6.6)	2.0 (3.7)	0.53	0.9 (3.1)	1.1 (2.7)	0.92
Bone mineral density of the femoral neck							
Integral BMD (mg/cm^3)	2104/2678	256 (50)	208 (41)	< 0.001	248 (50)	215 (40)	< 0.001
Trabecular BMD (mg/cm^3)	2104/2678	41 (42)	10 (34)	< 0.001	25 (44)	6 (36)	< 0.001
Cortical BMD (mg/cm^3)	2104/2678	544 (44)	529 (47)	< 0.001	534 (42)	523 (40)	0.003
Body composition							
Height (cm)	2104/2678	175.5 (6.2)	174.4 (6.7)	0.93	161.0 (5.7)	159.8 (5.7)	0.99
BMI (kg/m^2)	2104/2678	26.8 (3.8)	26.0 (4.1)	0.18	27.4 (4.8)	25.6 (4.7)	<0.001
Fat-free mass (kg)	1712/2219	64.0 (7.5)	61.6 (8.4)	0.15	46.0 (6.4)	43.4 (6.4)	<0.001
Fat mass (kg)	1712/2220	18.6 (7.0)	17.2 (7.0)	0.53	24.6 (7.4)	21.8 (7.4)	< 0.001
Thigh muscle area (cm^2)	1186/1558	119 (21)	107 (20)	0.02	85 (14)	77 (13)	< 0.001
Biomarkers							
25OHD (nmol/L)[2]	2104/2678	57.1 (24.4)	51.5 (25.4)	0.005	51.4 (23.2)	46.4 (21.8)	0.001
Hemoglobin (g/L)	2104/2678	14.1 (1.2)	13.7 (1.4)	0.08	13.2 (1.0)	12.9 (1.1)	0.03
Hemoglobin < 12.1 (g/L) (%)	2104/2678	7	15	0.08	15	23	0.02
Albumin (g/L)	1198/1502	41.2 (2.6)	40.0 (3.0)	0.02	40.9 (2.4)	40.5 (2.4)	0.41
Physical measurements							
Leg strength (N)	1945/2495	408 (108)	352 (184)	0.01	262 (76)	228 (67)	0.001
Leg strength < 10th percentile (%)		9.3	22.3	0.009	9.5	16.9	0.14
Grip strength (N)	1921/2467	389 (92)	363 (183)	0.95	236 (68)	217 (53)	0.20
Grip strength < 10th percentile (%)		9.5	19.0	0.24	9.5	15.0	0.30
Timed up and go (s)	2073/2654	12.1 (3.2)	13.8 (3.7)	0.001	12.4 (3.8)	13.3 (4.0)	0.46
Timed up and go > 15 s (%)		13	28	0.02	16	25	0.18
Balance forward-backward (cm)	1883/2424	9.1 (3.0)	7.4 (3.2)	0.001	7.9 (2.9)	7.1 (3.0)	0.69
Balance left-right (cm)	1883/2424	11.9 (3.2)	10.6 (3.7)	0.05	9.7 (3.3)	8.9 (3.0)	0.37
Other characteristics							
Alcohol more than 25 g (%)	2104/2678	27	19	0.25	11	10	0.64
Smoking (%)	2104/2678	12	11	0.62	13	13	0.11
Physical activity (%)	2090/2675	37	21	0.01	29	21	0.23
> 3 falls last 12 month (%)	2088/2675	1	2.6	0.02	2	4	0.07
Use of Calcium supplements, (%)	136/644	6	10	0.32	24	28	0.29
Use of Multi-vitamins, (%)	526/893	25	33	0.22	33	34	0.62
Taking cod liver oil, daily (%)[3]	1588/1911	77	66	0.008	73	69	0.30
On osteoporosis medication (%)[4]	7/113	0.3	2	0.03	4	8	0.02

[1] P value from independent samples' t test and chi-square test. Except for age all P values are adjusted for age as a continuous measure; [2] 25OHD = 25-hydroxy vitamin D; [3] A traditional food supplement containing ~ 800 IU of vitamin D; [4] Osteoporosis medication without estrogen

non-cases for females. That is, after adjustment for age and BMD significant differences in measures of body composition, serum 25(OH)D and leg strength were still clearly present. For males, significant differences in serum 25(OH)D, albumin, timed up and go test, and balance were also still present after adjustment for BMD, while differences in muscle thigh area and leg strength were no longer formally significant.

Table 3 shows the corresponding risk of fracture from the Cox regression analysis for all variables shown in Table 2.

Table 2 Differences in body composition, physical function, and general health status between fracture vs non-fracture participants ($N = 4782$)

	Males		Females	
	Fractures ($n = 114$) vs. no fractures ($n = 1990$)		Fractures ($n = 273$) vs. no fractures ($n = 2405$)	
	Adjusted for age	*Adjusted for age and BMD*[1]	*Adjusted for age*	*Adjusted for age and BMD*[1]
	Δ (95%CI)	*Δ (95%CI)*	*Δ (95%CI)*	*Δ (95%CI)*
Body composition				
BMI (kg/m^2)	− 0.5 (− 1.2, 0.2)	0.1 (− 0.7, 0.7)	**− 1.5 (− 2.1,− 0.9)**	**− 2.0 (− 1.6,− 0.4)**
Fat-free mass (kg)	− 1.8 (− 2.8, 0.4)	− 0.1 (− 1.7, 1.5)	**− 1.6 (− 2.5,− 0.8)**	**− 0.9 (− 1.8,− 0.1)**
Fat mass (kg)	− 0.5 (− 2.0, 1.0)	0.1 (− 1. 5, 1.6)	**− 2.0 (− 3.0,− 0.9)**	**− 1.2 (− 2.3,− 0.2)**
Height (cm)	0.1 (− 1.1, 1.2)	0.0 (− 1.1, 1.2)	− 0.0 (−0.7, 0.7)	0.1 (−0.6, 0.8)
Muscle thigh area (cm^2)	**− 5.6 (− 10.2,− 1.1)**	− 2.3 (− 6.8, 2.3)	**− 4.4 (− 6.5,− 2.3)**	**− 3.2 (− 5.3,− 1.14)**
Biomarkers				
25OHD (nmol/L)[2]	**− 5.9 (− 10.6,− 1.3)**	**− 4.6 (− 9.3, 0.0)**	**− 4.8 (− 7.8,− 1.9)**	**− 4.6 (− 7.5,− 1.6)**
Hemoglobin (g/L)	− 0.2 (− 0.4, 0.0)	− 0.2 (− 0.4, 0.1)	**− 0.2 (− 0.3,− 0.0)**	**− 0.1 (− 0.3,− 0.0)**
Albumin (g/L)	**− 0.9 (− 1.6,− 0.2)**	**− 0.8 (− 1.5,− 0.1)**	− 0.2 (− 0.62, 0.25)	− 0.2 (− 0.6, 0.3)
Physical measurements				
Leg strength (N)	**− 28 (− 49,− 7)**	− 19 (− 41, 2)	**− 16 (− 25,− 6)**	**− 14 (− 23,− 4)**
Grip strength (N)	− 1 (− 19, 18)	2 (− 17, 21)	− 5 (− 14, 3)	− 5 (− 13, 4)
Timed up and go (s)	**1.1 (0.5, 1.7)**	**1.0 (0.4, 1.6)**	0.2 (− 0.3, 0.6)	0.1 (− 0.3, 0.6)
Balance forward-backward (cm)	**− 1.0 (− 1.5,–0.4)**	**− 1.0 (− 1.6,− 0.4)**	− 0.1 (− 0.4, 0.3)	− 0.1(− 0.5, 0.3)
Balance left-right (cm)	− 0.6 (− 1.3, 0.0)	− 0.6 (− 1.2, 0.1)	− 0.2 (− 0.6, 0.2)	− 0.2 (− 0.6,0.2)

[1] BMD, integral bone mass density in femoral neck region. [2] 25OHD = 25-hydroxy vitamin D. Statistically significant estimates are shown in bold font

Overall, conclusions remained largely consistent, although some of the mean differences in Table 2 that were borderline significant (e.g. leg strength, hemoglobin, and balance left-right for males) or showed non-significant small differences (timed up and go test and grip strength) were formally significant in the Cox regression analyses (Table 3).

In the results presented in Tables 1, 2, and 3, a total of 1500 subjects, of which 183 were hip fracture cases, died during the follow-up period. Examining the influence of mortality during follow-up, we observed similar differences as presented in Table 1, and the same conclusions were reached when excluding those who died during the follow-up period (see Supplemental Table 2).

Discussion

In this cohort of 4782 older adults, we examined baseline characteristics of participants who later experienced a hip fracture during a mean follow-up time of 7.4 years versus those who did not. Participants who experienced hip fracture were generally older and frailer. After adjusting for age, the differences between the two groups can be summarized as reduced thigh muscle cross-sectional area, lower leg strength, and decreased physical function in terms of timed up and go and balance among males. Female cases, on the other hand, had lower BMI, less lean mass and fat mass, as well as reduced thigh muscle cross-sectional area and lower leg strength. These differences largely persisted after further adjustment for bone mineral density, suggesting that they may at least partly contribute to the risk of fracture independent of bone health.

Consistent with findings from other studies [3, 14, 33], hip fracture cases in our study scored lower in most baseline characteristics related to bone health and body composition. Apart from BMD, one of the most pronounced and consistent differences between hip fracture cases and non-cases for both sexes were observed for thigh muscle area and leg strength. Both measures are determinants of mobility and physical function in older adults. Several sex-specific differences were also observed. For example, after adjustment for age and BMD, differences in clinical biomarkers and physical function were more pronounced in males, while differences in body composition were more pronounced in females. Similar differences in body composition have been observed in other studies as well [3].

In line with our findings, the EPIDOS study [33] reported that both poor balance and physical function were, after adjustment for age and BMD, significantly associated with an increased risk of subsequent hip fractures. Two other studies [15, 34] have also suggested that limitations in physical functioning are related to increased hip fracture risk. With respect

Table 3 Associations between baseline characteristics and risk of hip fracture over the follow-up period ($N = 4782$)

	Males ($n = 2104$)		Females ($n = 2678$)	
	Adjusted for age	*Adjusted for age and BMD*[2]	*Adjusted for age*	*Adjusted for age and BMD*[2]
	HR (95%CI)[1]	*HR (95%CI)* [1]	*HR (95%CI)* [1]	*HR (95%CI)* [1]
Body composition				
BMI (kg/m^2)	0.86 (0.72, 1.04)	1.08 (0.88, 1.32)	**0.72 (0.64, 0.82)**	**0.80 (0.69, 0.92)**
Fat-free mass (kg)	0.85 (0.68, 1.08)	1.10 (0.87, 1.39)	**0.79 (0.70, 0.89)**	**0.87 (0.76, 1.00)**
Fat mass (kg)	0.97 (0.77, 1.22)	1.11 (0.89, 1.39)	**0.75 (0.64, 0.88)**	**0.85 (0.73, 0.99)**
Height (cm)	0.94 (0.78, 1.12)	1.12 (0.93, 1.35)	0.95 (0.85, 1.07)	1.02 (0.90, 1.17)
Muscle thigh area (cm^2)	**0.66 (0.51, 0.85)**	0.78 (0.59, 1.03)	**0.66 (0.57, 0.76)**	**0.66 (0.55, 0.81)**
Biomarkers				
25OHD (nmol/L) [3]	**0.72 (0.60, 0.88)**	0.82 (0.67, 1.00)	**0.87 (0.77, 0.98)**	**0.82 (0.72, 0.94)**
Hemoglobin (g/L)	**0.70 (0.59, 0.83)**	**0.78 (0.63, 0.95)**	**0.83 (0.75, 0.92)**	**0.84 (0.74, 0.95)**
Albumin (g/L)	**0.64 (0.51, 0.79)**	**0.68 (0.51, 0.90)**	0.83 (0.75, 1.02)	0.88 (0.73, 1.06)
Physical measurements				
Leg strength (N)	**0.56 (0.43, 0.72)**	**0.70 (0.53, 0.92)**	**0.75 (0.65, 0.87)**	**0.76 (0.65, 0.89)**
Grip strength (N)	**0.79 (0.64, 0.99)**	0.89 (0.70, 1.22)	**0.71 (0.61, 0.83)**	**0.73 (0.62, 0.85)**
Timed up and go (s)	**1.51 (1.33, 1.73)**	**1.53 (1.31, 1.79)**	**1.28 (1.16, 1.41)**	**1.25 (1.12, 1.40)**
Balance forward-backward (cm)	**0.69 (0.58, 0.83)**	**0.72 (0.60, 0.87)**	0.91 (0.80, 1.02)	0.92 (0.81, 1.04)
Balance left-right (cm)	**0.79 (0.66, 0.94)**	0.89 (0.74, 1.08)	0.92 (0.81, 1.04)	0.91 (0.79,1.03)

[1] Hazard ratios (HR) and 95% confidence intervals (CI). The hazard ratios reflecting change in risk 1-SD increase in exposure. [2] BMD, integral bone mass density in femoral neck region. Statistically significant estimates are shown in bold font. [3] 25OHD = 25-hydroxy vitamin D

to body composition, the Health ABC study found high subcutaneous fat thickness to be protective against hip fracture risk in both males and females [16]. This observation is also supported by more experimental work by Robinovitch et al. (1995). They showed that when applying the same impact, the measured peak force to the hip was reduced with increased trochanteric soft tissue thickness [34].

Concerning the sex-specific differences observed in our study, the higher incidence of hip fractures in females is, at least partly, explained by increased survival to older age, enhanced rate of bone loss after menopause [35], and smaller bone size compared with males [29]. These differences may explain the pattern observed in our study (Tables 2 and 3) with the protective role of body composition being more pronounced females [17, 35], while physical function, a marker of frailty and propensity of falls [36], being more pronounced for males who tend not to live as long as females.

In older adults' measures of physical function such as muscle thigh area, leg strength, timed up and go, and balance can be improved through resistance training and exercise. What impact such improvements may have on fracture risk must be examined in an intervention setting. When comparing cases and non-cases, absolute differences in the timed up and go test were stronger for males than females (Table 3). However, in terms of risk of fracture, a 1-SD increase in timed up and go was, after adjustment for age and BMD, significantly associated with around 50% and 25% higher risk of hip fracture in males and females, respectively. The strength of this association was similar or stronger to what was observed for other more advanced measures examined in our study. These results suggest that timed up and go test may be a relevant method to use when identifying older adults who are at increased fracture risk due to impaired functional mobility [37]. The advantages of using the timed up and go test are that it is non-invasive and less expensive than more advanced image techniques (e.g., DXA, CT, MRI).

The beneficial effect of sufficient vitamin D status on BMD is well established [38, 39], and both uses of vitamin D containing supplements and serum 25(OH)D status have been observed to be a predictor of bone health in this cohort [7, 40]. In our study, serum 25(OH)D also remained significantly different between hip fracture cases and non-fracture cases after adjustment for BMD, which is in line with findings from other studies [41, 42]. These associations may suggest a possible role of vitamin D in fracture prevention beyond bone density.

Regarding interpretation of our results, we presented both absolute mean differences in baseline characteristics of subjects who later (~ 5 years) experienced hip fractures compared with those who did not (Table 2) as well as examining the associations for these characteristics with time until fracture using Cox-regression analyses (Table 3). As the Cox regression analyses takes time to event into consideration, it provides a more direct and precise estimate of the underlying

association. The absolute differences, however, provide information on how different the two groups were in absolute term at baseline. Overall same conclusions were reached for both analyses but difference that were borderline significant in Table 2 (e.g., leg strength, hemoglobin, and balance left-right for males) reached formal significance when associations with time to event were examined (Table 3). For females, the timed up and go test and grip strength were significantly associated with increased risk of fractures (Table 3), while only modest and non-significant differences were observed for the mean differences at baseline (Table 2). In both cases the associations with risk of fractures appeared to be driven by few subjects with poor performance for these two measurements, which were slightly overrepresented among hip fracture cases (see Table 1).

Our study had several strengths, including its longitudinal design with a large number of participants who underwent a detailed, standardized clinical evaluation at baseline. After a relatively long follow-up period, hip fracture cases were extracted from clinical records and additionally verified. The study sample is representative of the elderly population in Iceland, which is reflected by a similar incidence of hip fractures compared with the general population [43]. Concerning limitations, despite the strength of the longitudinal design compared with other observational designs, these types of studies are still prone to biases due to improper confounder control and/or residual confounding. As a result, replication of our findings in another independent data source and further support from carefully designed interventions are needed.

Conclusions

Community-dwelling older adults who later experienced a hip fracture were generally older and frailer at the baseline evaluation, compared with those who did not subsequently suffer a fracture. After accounting for age and BMD, hip fracture cases had poorer baseline measures of physical function and/or body composition, which may at least partly contribute to the risk of falling, leading to fracture. A simple test of physical function, such as the timed up and go test, seems to compare favorably with more advanced methods using image analyses. In terms of preventive measures for hip fractures, more focus on improved physical function explored in intervention setting seems justified.

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Compliance with ethical standards All participants provided written informed consent. The study was approved by the Icelandic National Bioethics Committee (VSN: 00-063) and the National Institute on Aging Intramural Institutional Review Board (MedStar IRB for the Intramural Research Program, Baltimore, MD).

Conflicts of interest None.

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Paper II

ORIGINAL ARTICLE

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Serum 25-Hydroxy-Vitamin D Status and Incident Hip Fractures in Elderly Adults: Looking Beyond Bone Mineral Density

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ABSTRACT

Observational studies have consistently reported a higher risk of fractures among those with low levels of serum 25-hydroxyvitamin D (25(OH)D). Emerging evidence suggests that low serum 25(OH)D levels may increase the rate of falls through impaired physical function. Examine to what extent baseline measures of volumetric bone mineral density (vBMD), absolute bone mineral content (BMC), and markers of physical function may explain incident hip fractures in older adults with different serum levels of 25(OH)D. A prospective study of 4309 subjects (≥66 years) recruited between 2002 and 2006 into the Age, Gene/Environment Susceptibility-Reykjavik (AGES-Reykjavik) study. Hip fractures occurring until the end of 2012 were extracted from hospital records. Prevalence of serum 25(OH)D deficiency (<30 nmol/L), inadequacy (30–<50 nmol/L), and sufficiency (≥50 nmol/L) was 6%, 23%, and 71% for males; and 11%, 28%, and 53% for females, respectively. Female participants had ~30% lower absolute BMC compared to males. Serum 25(OH)D concentrations were positively associated with vBMD and BMC of the femoral neck and markers of physical function, including leg strength and balance. Those who had deficient compared to sufficient status at baseline had a higher age-adjusted risk of incidence hipfractures with hazard ratios (HRs) of 3.1 (95% confidence interval [CI], 1.9–5.2) and 1.8 (95% CI, 1.3–2.5) among males and females, respectively. When adjusting for vBMD and measures of physical function, the association was attenuated and became nonsignificant for males (1.3; 95% CI, 0.6–2.5) but remained significant for females (1.7; 95% CI, 1.1–2.4). Deficient compared to sufficient serum 25(OH)D status was associated with a higher risk of incident hip fractures. This association was explained by poorer vBMD and physical function for males but to a lesser extent for females. Lower absolute BMC among females due to smaller bone volume may account for these sex-specific differences. © 2021 American Society for Bone and Mineral Research (ASBMR).

KEY WORDS: HIP FRACTURES; VITAMIN-D STATUS; BONE MINERAL DENSITY; PHYSICAL FUNCTION; OLDER ADULTS

Introduction

Hip fractures are a significant cause of morbidity and mortality in the elderly.[1-5] Increased prevalence of hip fractures with age is to a large extent explained by the combination of deteriorating bone mass and increased propensity of falls.[6,7] Most hip fractures are caused by low-impact trauma that occurs after falling from standing height[6,8] with a gender ratio of around one male for every two to three females.[1,9,10]

Vitamin D deficiency is a known risk factor for osteoporosis[11] and observational studies have consistently reported positive associations between 25-hydroxyvitamin D (25(OH)D) with bone mineral density (BMD)[12,13] and a lower risk of incidence hip fractures.[14] However, the efficacy of vitamin D supplementation

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alone as a preventive measure has been somewhat uncertain with randomized controlled trials (RCTs) showing no clear benefits on hip fractures incidence.[15] Other RCTs have shown beneficial effects of vitamin D supplementation on the risk of falls.[16,17] Findings on a propensity for falls are in line with observational evidence suggesting a link between low serum 25(OH)D levels and poor physical function, including muscle strength[18] and balance.[19,20] The interpretation of these findings is complex, partly because many observational studies typically focus on individual markers of bone mass (ie, BMD and/or bone mineral content [BMC]) or different measures of physical function, but not both.

In a previous analysis on hip fractures from the longitudinal Age, Gene/Environment Susceptibility-Reykjavik (AGES-Reykjavik) study cohort ($n = 5764$), vitamin D deficiency, defined as serum 25(OH)D <30 nmol/L, was strongly associated with risk of incident hip fractures in both males and females.[21] However, the association's magnitude appeared larger than what could be explained by the modest positive association observed serum 25(OH)D and BMD. A later study, from the same cohort identified poor physical function as one of the main characteristics of hip fracture cases compared to non-cases.[22] Based on these observations, the aim of this study was to examine to what extent measurements of bone mass and markers of physical function might, when considered jointly, explain the inverse associations between serum 25(OH)D and incidence hip fracture in this older population.

Subjects and Methods

Study participants

The longitudinal Age, Gene/Environment Susceptibility-Reykjavik Study (AGES-Reykjavik) recruited between September 2002 to January 2006 a total of 5764 subjects aged ≥66 years. Recruitment into the AGES-Reykjavik study has been described in detail elsewhere.[23] The Icelandic National Bioethics Committee approved the AGES-Reykjavik study (VSN: 00-063) as did the National Institute on Aging Intramural Institutional Review Board (MedStar IRB for the Intramural Research Program, Baltimore, MD, USA).

For the present study, participants diagnosed with osteoporosis at baseline were excluded ($n = 522$). Another 933 subjects were excluded from analyses due to missing values on volumetric BMD (vBMD). The reason for missing values was that participants either could not attend the quantitative computed tomography (QCT) scanning or they were excluded from the scanning due to weight (>150 kg) or having undergone a previous hip replacement. Thus, 4309 subjects were included in present analyses. The reason for excluding those diagnosed with osteoporosis before recruitment was that those subjects would have been advised to take extra vitamin D supplementation, which might bias our estimates.

Baseline examination

During baseline examination when subjects were recruited into the study fasting serum blood samples were drawn and kept frozen at −80°C in the Icelandic Heart Associations (IHA) laboratory. Serum 25(OH)D(D_2 and D_3) was quantified by chemiluminescence (CLIA) using the LIAISON 25-OH Vitamin D Total assay (DiaSorin, Inc., Stillwater, MN, USA). The coefficient of variation was <7% based on a pool of frozen serum samples from AGES-Reykjavik and <13% when using LIAISON quality control standard.[21] The serum 25(OH)D concentrations as quantified by the LIASION assay were then re-scaled based on the procedures developed by the NIH-led Vitamin D Standardization Program (VDSP).[24] In brief the scaling was based on reanalyzes, for calibration, of serum 25(OH)D in a subset of bio-banked samples using a certified LC-MS/MS method, which is traceable to the National Institute of Standards and Technology (NIST) higher-order Reference Measurement Procedure.

At baseline examination participants were asked to bring all medication and supplements used during the previous 2 weeks to the clinic to be recorded. During the examination, body weight was measured with participants wearing light underwear and height measured with a calibrated stadiometer. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. Bioelectric impedance analysis (BIA) was used to measure fat mass and fat-free mass. Participants were also interviewed and asked to self-report on sociodemographic characteristics and health related behaviors, physical activity, and specific physician-diagnosed diseases and conditions.

Integral vBMD (mg/cm^3), bone volume (BV, cm^3), and BMC (g) of the femoral neck was quantified using QCT scanning (1-mm slices) as described.[7,23] A single axial QCT-section through the right mid-thigh was used to quantify the geometry of mid-thigh, cross-sectional muscular area, and subcutaneous tissue.[25]

During the clinical examination, various measures of balance, muscular strength and physical function were also recorded. For the balance test,[26] participants were asked to stand with feet separated from each other (the exact position of the V-feet placement in a relaxed upright position on a force [postural] platform), with hands beside the body. The test consisted of looking at a computer monitor with a picture of five boxes on the screen, one in the center and one box in each of the screen's four corners. The participant was then asked to move his body without moving the feet toward the box in each corner without losing balance or taking an extra step from the standing position. The maximum distance achieved was then recorded. Muscle strength was assessed as a maximal isometric strength of the dominant leg and hand of the individual sitting in an adjustable dynamometer chair (Good Strength, Metitur Ltd., Palokka, Finland). Knee extension was measured with the knee angle at 60 degrees and the ankle fastened by a belt to a strain-gauge system. Handgrip strength was measured with a dynamometer fixed to the arm with the elbow flexed at 90 degrees and using the same instructions and methods as for the lower limb.[27] Physical function was assessed using a time-up-and-go (TUG) test and a 6-m gait speed test. TUG measures the time it takes to stand up from a chair without arms, walk 3 m, return, and sit down without using the upper limb for assistance.[28,29] The 6-m gait speed test was assessed by recording the time it took using a stopwatch, to walk 6 m as fast as the participant could achieve.[30]

Assessment of hip fractures

Information on hip fractures, defined according to the International Classification of Diseases and Related Health Problems, 10th Revision (ICD-10) codes S72.0, S72.1, and S72.2, were extracted from hospital records, an independent radiologist confirmed the fracture type.[31] The extracted information covered all events occurring from participants enrollment into the study (September 2002 to January 2006) until December 31, 2012.

Statistical analyses

Statistical analyses were performed using SPSS for Windows version 25.0 (IBM Corp., Armonk, NY, USA). In all analyses level of

significance was set at $p < 0.05$ (two-sided). Mean and standard deviation (SD) were used to describe continuous normally distributed variables. The *t* test or *F* test were used to compare differences across two or more groups, respectively. The normality assumption was verified by visual inspection of histograms and quantile-quantile plots. Percentages were used to describe dichotomous variables, and the chi-square test was used to compare proportion across groups. All analyses were performed for males and females separately.

Baseline serum 25(OH)D status was divided into three categories according to definitions proposed by the Institute of Medicine (IOM)[32] based on their review on the relationship between serum 25(OH)D and calcium absorption. On the basis of this review, IOM defined vitamin D deficiency as serum 25(OH)D < 30 nmol/L, inadequacy as serum 25(OH)D 30 to <50 nmol/L and sufficiency as serum 25(OH)D as ≥50 nmol/L. These three categories were used in our primary analyses focusing on possible adverse associations that might occur at low serum 25(OH)D concentrations. Possible benefits occurring at higher concentrations were given less weight because IOM has previously concluded that "serum 25OHD concentrations above 75 nmol/L (30 ng/mL) are not consistently associated with increased benefit," which is also consistent with our previous findings on vBMD and incident hip fractures in this cohort.[21]

Using linear regression analyses we compared cross-sectionally differences in baseline measures of bone mass (ie, vBMD, BMC, and BV), measures of body composition and objective measures of physical function across the three categories of serum 25(OH)D in age-adjusted analyses using the sufficient category as the reference. Based on these results we were able to identify which baseline measures of bone mass, body composition and physical function were associated with serum 25(OH)D status that might contribute to later risk of incidence hip fractures. Acknowledging that different views exist on categorization of serum 25(OH)D status, all associations were also evaluated by modeling serum 25(OH)D as a continuous term in a separate regression model. The effect estimate reported reflects the change in the outcome variable per 10-nmol/L increase in serum 25(OH)D. The *p* value for this regression slope is referred to in our tables as *p* for trend.

Based on these analyses, we then examined prospectively the relationship between serum 25(OH)D, modeled as categorical and continuous term, with incidence hip fractures using various degrees of statistical adjustment. For these analyses we used a Cox proportional hazard model using the time from baseline measure (September 2002 to January 2006) and until hip fracture occurred or end of follow-up (December 31, 2012), censuring those who died during the follow-up period. For covariate

Table 1. Baseline Characteristics of AGES-Reykjavik Male Participants According to Serum 25(OH)D Concentrations at Recruitment

Males ($n = 2053$)	<30 nmol/L ($n = 125$)[a]	30 to <50 nmol/L ($n = 465$)[a]	≥50 nmol/L ($n = 1463$)[a]	p[b]
Age (years), mean ± SD	77.0 ± 6.1	76.3 ± 5.5	76.6 ± 5.3	0.47
Body composition, mean ± SD				
Height (cm)	175 ± 6.5	175 ± 6.2	176 ± 6.1	0.02
Weight (kg)	84 ± 15.3	83 ± 13.8	83 ± 12.8	0.45
BMI (kg/m^2)	27.25 ± 4.6	27.19 ± 3.9	26.69 ± 3.6	0.02
% Total of body fat	22.9 ± 6.0	22.9 ± 5.3	21.5 ± 5.4	<0.001
QCT variables for the femoral neck, mean ± SD				
Integral volumetric BMD (mg/cm^3)	240 ± 50	251 ± 49	257 ± 50	<0.001
Bone mineral content (g)	4.91 ± 1.3	4.99 ± 1.9	5.1 ± 1.2	0.01
Bone volume (cm^3)	20.52 ± 3.8	20.08 ± 3.8	20.25 ± 3.9	0.50
Physical function, mean ± SD				
Muscle area of the right thigh (cm^2)	113 ± 22	116 ± 23	120 ± 20	0.003
Subcutaneous fat in right thigh (cm^2)	45 ± 18	49 ± 23	42 ± 19	<0.001
Leg strength (N)	390 ± 115	399 ± 115	409 ± 113	0.09
Grip strength (N)	378 ± 90	381 ± 95	391 ± 101	0.12
Timed Up and Go test (seconds)	12.6 ± 4.0	12.4 ± 3.3	12.1 ± 3.1	0.09
6 m gait speed test (seconds)	4.8 ± 1.1	4.7 ± 1.2	4.5 ± 0.9	<0.001
Balance (cm) backward-forward	8.7 ± 2.8	8.7 ± 3.0	9.1 ± 3.0	0.02
Other characteristics				
Charlson-score, mean ± SD	1.9 ± 3.4	1.6 ± 3.6	1.4 ± 7.5	0.67
Number of medications, mean ± SD	4.4 ± 3.4	4.3 ± 3.2	4.3 ± 3.1	0.99
Physical inactivity, *n* (%)[c]	105 (87.5)	332 (72.5)	867 (60.0)	<0.001
Current use of cod liver oil or other vitamin D–containing supplements, *n* (%)	48 (40)	286 (61.9)	1252 (86.7)	<0.001
Parkinson's disease, *n* (%)	1 (0.8)	13 (2.8)	15 (1.0)	0.02
Epilepsy, *n* (%)	3 (2.5)	5 (1.1)	10 (0.7)	0.11
Passed out, fainted, or lost consciousness in the past 12 months, *n* (%)	8 (6.6)	16 (3.5)	48 (3.3)	0.18

BMD = bone mineral density; QCT = quantitative computed tomography; SD = standard deviation.

[a]The median serum 25(OH)D concentration in each category was 25, 41, and 66 nmol/L, respectively.

[b]*F* test for continuous variables and chi-square test for dichotomous variables.

[c]Physical inactivity here is defined as those reporting to never, rarely, or occasionally to engage in physical activity (and those reporting frequent or daily activity were considered physically active).

adjustment, we used four different models. Model 1 adjusted for age and BMI; model 2 additionally adjusted for vBMD of the femoral neck; model 3 included age, BMI, leg strength, the TUG test, and the balance test; and model 4 was the fully adjusted model with all covariates in models 2 and 3. The objective of these analyses was to identify to what extent any associations with hip fractures could be explained by baseline status of vBMD (model 2) or markers of physical function (model 3) and the combined contribution of the two (model 4). For these adjustments missing covariate values were imputed ($n = 5$) as implemented in the missing value module in SPSS.

Results

The mean age ± SD of male ($n = 2053$) and female ($n = 2256$) participants was 76.5 ± 5.4 and 76.0 ± 5.6 years, respectively. For males, the prevalence of vitamin D deficiency (<30 nmol/L), inadequacy (30 to <50 nmol/L), and sufficiency (≥50 nmol/L) was 6%, 23%, and 71%, respectively. The corresponding numbers for females were 11%, 28%, and 53%, respectively. For those interested in the prevalence of higher concentrations, a total of 18% and 11% had serum 25(OH)D >75 nmol for males and females, respectively.

The unadjusted baseline characteristics of male and female participants across categories of serum 25(OH)D status are shown in Tables 1 and 2, respectively. For both sexes higher serum 25(OH)D status was significantly associated with lower BMI and percentage body fat and better performance in different tests measuring physical function. As expected, those with higher serum 25(OH)D were more physically active and more likely to use vitamin D supplements.

For both sexes having sufficient serum 25(OH)D status was also associated with higher vBMD in the femoral neck, BMC, but not BV (Tables 1 and 2). In terms of gender specific differences, absolute BMC (ie, vBMD × BV) was ~30% lower in females (3.7 g) compared to males (5.1 g), which was largely explained by smaller bone volume in females (see Supplemental Table S1). The lower BMC among females appeared to explain why the prevalence of incident hip fractures were around two times higher in females (9.4%) compared to males (5.3%). This conclusion was based on the observation that when BMC was divided into deciles for males and females separately the prevalence of incident hip fractures was highest (~15%) at very low BMC (<3.0 mg) where only females were represented but lowest (~2%) at high BMC (>5.0 mg) where only males were represented (see Fig. 1). Similar prevalence of incident hip fractures (~8% to 9%) was, however, observed in both sexes in the interval

Table 2. Baseline Characteristics of AGES-Reykjavik Female Participants According to Serum 25(OH)D Concentrations at Recruitment

Females ($n = 2256$)	<30 nmol/L ($n = 236$)[a]	30 to <50 nmol/L ($n = 623$)[a]	≥50 nmol/L ($n = 1397$)[a]	p[b]
Age (years), mean ± SD	76.9 ± 6.0	75.8 ± 5.2	76.0 ± 5.7	0.04
Body composition, mean ± SD				
Height (cm)	160.4 ± 5.2	161.0 ± 5.5	161.3 ± 5.7	0.08
Weight (kg)	74.2 ± 16.1	73.5 ± 14.2	70.2 ± 12.1	<0.001
BMI (kg/m^2)	28.7 ± 5.8	28.3 ± 5.1	27.0 ± 4.4	<0.001
% Total of body fat	34.8 ± 5.5	35.0 ± 5.2	33.8 ± 5.0	<0.001
QCT variables for the femoral neck, mean ± SD				
Integral volumetric BMD (mg/cm^3)	242 ± 47	251 ± 53	252 ± 48	0.02
Bone mineral content (g)	3.49 ± 0.8	3.66 ± 0.9	3.70 ± 0.8	0.003
Bone volume (cm^3)	14.5 ± 2.7	14.7 ± 2.8	14.8 ± 2.7	0.31
Physical function, mean ± SD				
Muscle area of the right thigh (cm^2)	84 ± 16	85 ± 15	85 ± 14	0.59
Subcutaneous fat in right thigh (cm^2)	104 ± 46	110 ± 45	101 ± 41	0.001
Leg strength (N)	238 ± 73	261 ± 75	268 ± 77	<0.001
Grip strength (N)	228 ± 59	235 ± 58	239 ± 75	0.08
Timed Up and Go test (seconds)	13.7 ± 4.3	12.6 ± 3.6	12.1 ± 3.6	<0.001
6 m gait speed test (seconds)	5.4 ± 1.2	5.2 ± 1.4	5.0 ± 1.1	<0.001
Balance (cm) backward-forward	7.0 ± 3.1	7.7 ± 3.0	8.0 ± 2.9	<0.001
Other characteristics				
Charlson-score, mean ± SD	1.1 ± 2.3	0.9 ± 3.3	0.8 ± 3.0	0.44
Number of medications, mean ± SD	4.3 ± 3.1	4.2 ± 3.0	4.2 ± 3.1	0.95
Physical inactivity, n (%)[c]	197 (84.9)	473 (76.8)	941 (68.3)	<0.001
Current use of cod liver oil or other vitamin D–containing supplements, n (%)	107 (46.3)	395 (64.1)	1176 (85.2)	<0.001
Parkinson's disease, n (%)	4 (1.7)	7 (1.1)	5 (0.7)	0.03
Epilepsy, n (%)	7 (3.0)	6 (1.0)	8 (0.6)	0.002
Passed out, fainted, or lost consciousness in the past 12 months, n (%)	7 (3.0)	25 (4.0)	55 (4.0)	0.75

BMD = bone mineral density; QCT = quantitative computed tomography; SD = standard deviation.

[a]The median serum 25(OH)D concentration in each category was 25, 41, and 64 nmol/L, respectively.

[b]F test for continuous variables and chi-square test for dichotomous variables. Number of missing in values for the <30, 30 to <50, and the >50 nmol/L categories.

[c]Physical inactivity here is defined as those reporting to never, rarely, or occasionally to engage in physical activity (and those reporting frequent or daily activity were considered physically active).

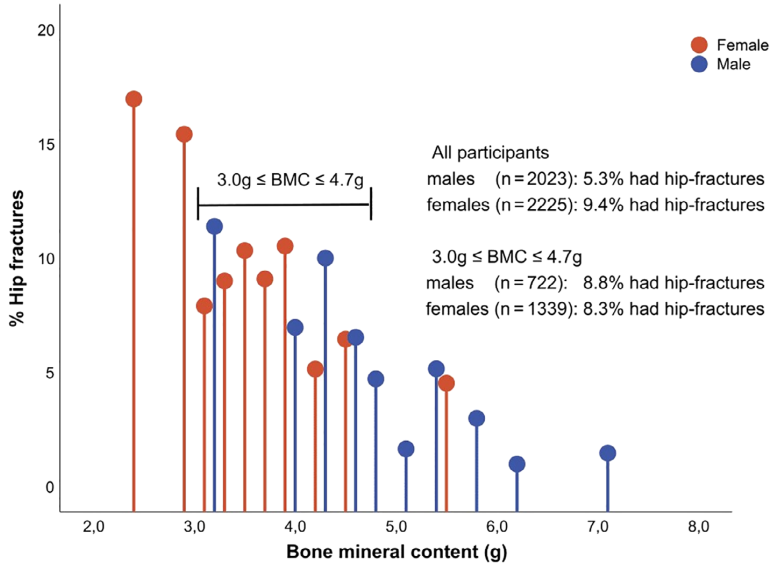

Fig 1. Prevalence of incident hip fractures in relation to BMC in males and females. The distribution for BMC was divided into deciles for males and females separately and the prevalence of incident fracturs plotted using the median value in each decile. In the area where both males and females have substantial overlap (3.0 ≤ BMC ≤ 4.7 g) similar prevalence of hip fractures (∼8%–9%) is observed, whereas the lower overall prevalence among males (5.3%) compared to females (9.4%) appears to be driven by higher BMC among men. BMC = bone mineral content.

(3.0–4.7 mg) where there was high degree of overlap in the sex-specific distribution of BMC.

Table 3 shows the age-adjusted mean differences in the characteristics reported in Tables 1 and 2 across the three categories of vitamin D status. Overall, a similar pattern was observed for males and females. For example, using those with sufficient status (≥50 nmol/L) as reference, those with inadequate (30 to <50 nmol/L) and deficient status (<30 nmol/L) had higher BMI, higher percentage body fat, lower leg strength and grip strength, and they performed worse on the TUG test, gait speed, and the balance test. However, in absolute terms, the mean change in these measures varied between the two sexes. For males, there was, for example, a relatively strong decrease in vBMD and BMC going from sufficient to deficient serum 25(OH) D status. A similar association was observed for females, but the absolute decrease in vBMD and BMC was smaller. In terms of sex-specific differences, the cross-sectional muscle area of the right mid-thigh decreased significantly going from sufficient to deficient serum 25(OH)D status among males, whereas no such association was observed for females. Supplemental Table S2 shows the association between serum 25(OH)D modeled as continuous linear variable with the same outcome measures as shown for the categorical analyses in Table 3. The conclusions reached were similar when comparing the categorical versus continuous measurement of serum 25 (OH)D status.

Finally, Table 4 shows the association across the three categories of baseline serum 25(OH)D status and incident hip fractures over a mean follow-up time of 7.5 ± 2.4 years. After adjustment for age and BMI (model 1), males who were serum 25(OH)D-deficient at baseline compared to those with sufficient status had around threefold higher risk of incidence hip fractures (hazard ratio [HR] 3.1; 95% confidence interval [CI], 1.9–5.2). After additional adjustment for either vBMD (model 2) or measures of physical function (eg, leg strength, balance, and TUG in model 3) the association with hip fractures was substantially reduced but still significant. However, when adjusting for all measures combined (model 4), the association with hip fractures became nonsignificant (HR 1.3; 95% CI, 0.6–2.5). For females, the association with hip fractures in model 1 was, when comparing those with deficient versus sufficient serum 25(O)D status, considerably weaker compared to males (HR 1.8; 95% CI, 1.3–2.5). In contrast to males, adjustment for both vBMD and physical function measures did not influence the effect estimates for hip fractures for females in the fully adjusted model 4 (HR 1.7; 95% CI, 1.1–2.4). Further adjustment for disease conditions that might contribute to higher propensity of fall such as Parkinson's disease, epilepsy, or having passed out, fainted, or lost consciousness over the past 12 months only minimally influenced the estimates (<5%) and same conclusions as for model 4 were reached (data not shown).

Discussion

In a cohort of 4309 participants from AGES-Reykjavik study, we found that those with deficient (<30 nmol/L) and to lesser extent inadequate (30 to <50 nmol/L) vitamin D status had lower vBMD and BMC of the femoral neck, but higher BMI and percent body fat compared to those sufficient (≥50 nmol/L) status. Similarly, subjects with deficient levels also had worse physical function as measured by leg strength and other objective, functional tests; these differences were observed in both sexes. Having deficient serum 25(OH)D status at baseline was also strongly

Table 3. Mean Difference in Baseline Measures of Body Composition, Volumetric Bone Mineral Density and Content, and Physical Function According to Baseline Serum 25(OH)D Status Using ≥50 nmol/L as a Reference Category With All Comparisons Age-Adjusted

Parameter	Serum 25(OH)D (nmol/L)[a]	Males ($n = 2053$) mean difference (95% CI)[b]	Females ($n = 2256$) mean difference (95% CI)[b]
Body composition			
BMI (kg/m^2)	<30	0.6 (−0.1 to 1.3)	1.9 (1.2 to 2.5)
	30 to <50	0.5 (0.1 to 0.9)	1.3 (0.9 to 1.8)
	≥50	Referent	Referent
	p for trend[c]	<0.001	<0.001
Muscle area in the right thigh (cm^2)	<30	−6.1 (−10.5 to −1.8)	−1.0 (−3.2 to 1.3)
	30 to <50	−4.4 (−6.9 to −1.9)	−0.9 (−2.5 to 0.7)
	≥50	Referent	Referent
	p for trend	0.008	0.68
Subcutaneous fat in right thigh (cm^2)	<30	2.7 (−2.1 to 7.5)	4.1 (−3.3 to 11.5)
	30 to <50	6.1 (3.3 to 8.8)	9.1 (3.8 to 14.4)
	≥50	Referent	Referent
	p for trend	<0.001	0.02
Body fat (%)	<30	1.4 (0.3 to 2.5)	1.2 (0.4 to 2.0)
	30 to <50	1.3 (0.7 to 2.0)	1.2 (0.7 to 1.7)
	≥50	Referent	Referent
	p for trend	<0.001	<0.001
Integral volumetric BMD (mg/cm^3)	<30	−17 (−26 to −8)	−7 (−14 to −1)
	30 to <50	−7 (−12 to −2)	1 (−5 to 4)
	≥50	Referent	Referent
	p for trend	<0.001	0.003
Bone mineral content (cm^3)	<30	−0.22 (−0.44 to 0.01)	−0.17 (−0.28 to −0.06)
	30 to <50	−0.16 (−0.29 to −0.04)	−0.05 (−0.12 to 0.03)
	≥50	Referent	Referent
	p for trend	<0.001	<0.001
Physical function			
Time up and go (second)	<30	0.40 (−0.18 to 0.97)	1.41 (0.93 to 1.90)
	30 to <50	0.36 (0.04 to 0.69)	0.53 (0.20 to 0.86)
	≥50	Referent	Referent
	p for trend	0.004	<0.001
6 m gait speed (second)	<30	0.3 (0.1 to 0.5)	0.4 (0.2 to 0.5)
	30 to <50	0.2 (0.1 to 0.3)	0.2 (0.1 to 0.4)
	≥50	Referent	Referent
	p for trend	<0.001	<0.001
Leg strength (N)	<30	−15.9 (−35.1 to 5.0)	−26.3 (−36.3 to −16.3)
	30 to <50	−13.0 (−24.3 to −1.7)	−9.0 (−15.8 to −2.3)
	≥50	Referent	Referent
	p for trend	0.13	<0.001
Grip strength (N)	<30	−10.2 (−27.5 to 7.2)	−7.8 (−17.1 to 1.4)
	30 to <50	−11.5 (−21.4 to 1.6)	−4.4 (−10.8 to 1.9)
	≥50	Referent	Referent
	p for trend	0.05	0.22
Balance, backward – forward (cm)	<30	−0.37 (−0.92 to 0.18)	−0.8 (−1.2 to −0.4)
	30 to <50	−0.52 (−0.82 to −0.21)	−0.3 (−0.5 to 0.2)
	≥50	Referent	Referent
	p for trend	<0.001	0.001

BMD = bone mineral density; CI = confidence interval.

[a]The median serum 25(OH)D concentration in each category was 25, 41 and 66 nmol/L for males and 25, 41 and 66 nmol/L for females, respectively.

[b]Mean difference from the referent, confidence intervals strictly below or above the zero reflect significant differences at alpha = 5%.

[c]Linear trend test result obtained by modeling serum 25(OH)D as a continuous variable in the regression model, see the corresponding estimate (linear slope) in Supplemental Table S2.

Table 4. Associations Between Baseline Serum 25(OH)D Concentrations and Incidence Hip Fractures

	Males			Females		
Serum 25(OH)D (nmol/L)[a]	*n*	HR (95% CI)	*p*	*n*	HR (95% CI)	*p*
Model 1[b]						
<30	15/125	3.08 (1.85–5.15)	<0.001	35/236	1.80 (1.29–2.50)	0.001
30 to <50	28/465	1.28 (0.85–1.93)	0.24	62/623	1.14 (0.88–1.48)	0.32
≥50	66/1463	1.00		113/1397	1.00	
p for trend[c]		0.002			<0.001	
Model 2[d]						
<30		1.98 (1.12–3.50)	0.02		1.82 (1.28–2.59)	0.001
30 to <50		1.21 (0.78–1.89)	0.40		1.26 (0.97–1.67)	0.08
≥50		1.00			1.00	
p for trend		0.04			<0.001	
Model 3[e]						
<30		2.53 (1.39–4.62)	0.002		1.63 (1.12–2.37)	0.01
30 to <50		1.11 (0.69–1.77)	0.68		1.22 (0.92– 1.62)	0.17
≥50		1.00			1.00	
p for trend		0.03			<0.001	
Model 4[f]						
<30		1.26 (0.62–2.54)	0.52		1.65 (1.12–2.44)	0.01
30 to <50		1.17 (0.72–1.91)	0.54		1.31 (0.98–1.76)	0.07
≥50		1.00			1.00	
p for trend		0.20			0.004	

Time from baseline measurement of serum 25(OH)D (September 2002 to January 2006) and until hip fracture occurred or end of follow-up (December 31, 2012), censuring those who died during the follow-up period.

HR = hazard ratio; CI = confidence interval.

[a]The median serum 25(OH)D concentration in each category was 25, 41, and 69 nmol/L for males and 25, 41, and 66 nmol/L for females, respectively.

[b]Model 1: Adjustment for age and BMI.

[c]Linear trend test (chi-square) obtained by modeling serum 25(OH)D as continuous variable in the regression model, see the corresponding estimate (linear slope) in Supplemental Table S3.

[d]Model 2: Model 1 with additional adjustment for volumetric bone mineral density.

[e]Model 3: Model 1 with additional adjustment for leg strength, balance and TUG.

[f]Model 4: All covariates in models 2 and 3.

associated with incidence hip fracture in both sexes. However, this association was more pronounced in males where it was explained by lower vBMD and poorer physical function among those with deficient vitamin D status. Neither of these factors could fully explain the association for females.

Associations between deficient serum 25(OH)D status and incidence of hip fractures have previously been reported in this cohort, although with slightly shorter follow-up time.[21] As in this study, the absolute difference in vBMD between those having deficient (<30 nmol/L) versus sufficient (≥50 nmol/L) status was approximately −8 mg/cm^3 in females and −15 mg/cm^3 in males. This effect size corresponds to around 15% and 30% of the SD for vBMD, respectively. A previous meta-analysis (n ~ 40.000) of 12 prospective cohorts found that the relative risk of hip fractures for both sexes increased about twofold per 1 SD decrease in BMD.[33] The observed effect size for vBMD in our study would therefore be too small to explain an approximately threefold and approximately twofold increase in the risk of fractures among males and females, respectively, among those with deficient versus sufficient status. This observation combined with emerging evidence on the effects of vitamin D supplementation on the rate of falls[34] and some evidence linking low serum 25(OH)D status with poor muscle strength[17] prompted re-analyses of our data.[21]

In our cross-sectional analyses deficient and, to lesser extent, inadequate serum 25(OH)D status was associated with lower leg and grip strength, poorer balance, and worse performance in both the TUG test and gait speed. In some cases, significance was not always reached for the deficient group, which may be explained by fewer subjects in that category. Interestingly, these associations were observed in both males and females and, if anything, with a slightly larger effect size among females (eg, lower leg strength, balance, and TUG test).

Our findings on the associations between serum 25(OH)D status and balance are in line with results from RCTs. As an example, in a study of 160 Brazilian women aged 50 to 65 years old who were vitamin D–deficient (serum 25(OH)D <30 nmol/L) a supplementation over 9 months resulted in around a threefold reduction in the occurrence of falls and improvement in balance (measured by body sway) compared to controls.[35] Another RCT of 242 elderly (mean age 77 years) women from Germany and Austria (mean serum 25(OH)D ~55 nmol/L) a 12-month supplementation with vitamin D and calcium significantly reduced the occurrence of falls and improved balance compared to calcium supplementation alone.[19] In that study, a significant modest difference in both leg (quadriceps) strength and performance in the TUG test was observed compared to controls. Still, results from RCTs examining the effect of vitamin D supplementation on muscle strength have not always been consistent[36] and remain controversial, including an increased propensity of falls observed at high (50,000 UI) supplemental doses.[37]

In our longitudinal analyses for hip fracture risk we saw that deficient vitamin D status was associated with increased incidence hip fracture. After adjustment for vBMD the HR for males was reduced from ~3 to 2 when comparing those with deficient (<30 nmol/L) status to those with sufficient status (≥50 nmol/L), but the overall

association was still significant. Further adjustment for leg strength, balance and the TUG test reduced the association further (HR ~1.3), and the association became nonsignificant. Based on these analyses it can be concluded that baseline markers of physical function and vBMD fully accounts for the association between deficient serum 25(OH)D and hip fractures in males. In contrast, adjustment for vBMD and markers of physical function had limited influence on the association for females.

Inconsistent associations for males and females may seem contradictory, particularly given the similar associations observed between baseline serum 25(OH)D status with lower bone mass (ie, vBMD and BMC) and markers of physical function in both sexes. However, it is worth noting that hip fractures that occur due to low-impact falls are primarily explained by poor bone strength in combination with an increased propensity for falls, as reported in a recent study.[1] Although functional measures such as the balance test, leg strength and the TUG test measure physical function that may be predictive of the rate of falls,[20] these tests are not a direct measure per se. Other aspects, such as cognitive status and reflexes, also play a role.[38,39] If those factors, not accounted for in our study, differ between males and females at older ages, this may explain why adjustment for measures of physical function was influential for males but not females.

Furthermore, although vBMD at baseline did not differ substantially between the two sexes (see Tables 1 and 2), females had ~30% lower BMC (ie, vBMD $\times$ BV) that likely reflects considerably lower bone strength compared to males. Lower BMC is to large extent explained by smaller BV among females. The resulting lower BMC in females is only modestly correlated with weight and may on its own explain why the prevalence of hip fractures, as observed our study and reported by others,[40,41] is around two to three times higher in females compared to males at an older age. This conclusion is supported by our observation that in the interval where BMC overlapped in both sexes the prevalence of incident hip fractures was comparable (see Fig. 1). In summary, it is plausible that the lower BMC due to smaller BV in females compared to males may explain why adjustment of vBMD and physical function did not account for the association observed for hip fractures in females as compared to males in our study. This explanation seems plausible if deficient serum 25(OH)D status is truly related to an increased rate of falls as results from some RCTs seem to suggest.[34] The limitations of this explanation, however, is that we lacked direct measures of falls in our study.

Although our cross-sectional findings on poorer bone mass and reduced physical function in males and females are supported by findings from intervention studies,[19,34] our study's observational nature means that our findings are prone to bias, including reverse causation and confounding. In terms of reverse causation, we excluded all subjects who had osteoporosis at baseline because these subjects are generally given an extra dose of vitamin D supplements after diagnosis. Concerning other limitations, it is well established that seasons and other external factors can have strong influence serum 25(OH)D status. In that context one of the main limitation of our study is that we only had a single baseline measurement of serum 25(OH)D. The resulting misclassification, based on our single measure, may have been particularly relevant for those classified as being deficient.[42] Furthermore we also acknowledge the limitations of some of our baseline measures, such as not having information on previous physical activity and relying on bioelectric impedance to assess fat and fat-free mass, which is not the gold standard.

In terms of study strengths, it has been estimated that during follow-up ~97% of all hip fractures occurring in our study population were captured by the extracted hospital records.[31] Another strength is the use used QCT to quantify bone mass (ie, vBMD, BMC), which gives a three-dimensional direct measure of volume. Our results also clearly demonstrate that both deficient and, to lesser extent, inadequate serum 25(OH)D status is associated with poor performance in functional tests measuring physical function and with poorer vBMD and BMC. Inclusion of these baseline measures provides a more nuanced interpretation of the association between serum 25(OH)D and incidence hip fractures observed in our study.

Additionally, our findings highlight areas of research that require further consideration. First, although observational studies have consistently shown inverse association between serum 25(OH)D status and incident hip fractures[33] results from RCTs have been suggestive but inconclusive.[15,43] The large heterogeneity across interventions may to some extent be explained by how subjects are recruited in terms of age and baseline vitamin D status. The latter has not been given much consideration, but those trials which have recruited subjects with deficient status have generally shown consistent and larger improvements in reducing risk of fractures,[43,44] BMD,[12,45] and measures of physical function.[18,19,35] Our observational study on data derived from a large sample of community dwelling residents highlights this point; that those with deficient status generally performed worse compared to those with sufficient serum 25(OH)D status. In addition, given the small BV in females compared to males, our study also highlights the need to focus on the absolute BMCand not just BMD when interpreting gender-specific differences in hip fractures. This issue has been previously raised,[41,46] but is often not given much attention.

In summary, our findings suggest that deficient (<30 nmol/L) and to lesser extent inadequate (30 to <50 nmol/L) vitamin D status is associated with poorer physical function and bone mass (ie, vBMD and BMC) compared to those with sufficient status (≥50 nmol/L). These factors could explain why baseline vitamin D status was inversely associated with incidence hip fractures in males. The association with incident hip fractures in females appeared more nuanced but could be related to lower absolute BMC in females compared to males. Intervention studies focusing on the efficacy of vitamin D supplementation should ideally focus on those with deficient status because this is where most benefits could be expected.

Disclosures

All authors state that they have no conflicts of interest.

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Authors' roles: Study design: SSS, GS and TIH. Data analysis: SSS, AR and TIH. Data interpretation: SSS, AR and TIH. Drafting manuscript: SSS, AR and TIH. Revising manuscript content: SSS, AR, MFC, HE and TIH. Approving final version of manuscript: SSS, AR, IH, LJL,MFC,KS,VG, HE, GS,LS and TIH. SSS takes responsibility for the integrity of the data analysis.

Peer Review

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Data Availability Statement

Research data are not shared but access may be granted upon request according to established procedures for the AGES-Reykjavik Study.

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Paper III

Title: Milk intake and hip fracture incidence in community-dwelling old Icelandic adults.

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Abstract (Aiming for publication in Osteoporosis International).

This study describes associations between milk intake and hip fracture risk in older Icelanders. The data indicate that increased milk consumption is related to lower hip fracture risk.

Background: Hip-fracture can have a severe effect on the life of older adults. Health authorities recommend milk intake for better bone health. However, previous studies addressing this issue have been conflicting.

Methods: This prospective study included 4614 subjects (mean age 76 years) recruited between 2002 to 2006 into the Age, Gene/Environment Susceptibility-Reykjavik (AGES-Reykjavik) study. Information on hip fractures occurring between recruitment and end of 2012 were extracted from hospital records.

Results: A total of 14% of participants reported milk intake <0.5 servings/day (the lowest category), and 22% of the participants consumed at least two milk servings/day (highest category). Milk consumption was positively related to the volumetric bone mineral density at baseline with a sex- and age-adjusted difference of 9.7±2.5 mg/cm^3 between the highest compared to lowest milk intake categories (P<0.001). During the follow-up time, 7.4% of participants had a hip fracture, and we observed an increased risk of incident hip fractures in the lowest compared to the highest milk intake category with a hazard ratio of 1.46 (95%CI: 1.01-2.11) in the fully adjusted model. Further analysis indicated a linear relationship between milk intake and fracture risk (P-value for linear trend <0.001).

Conclusion: Milk intake is associated with a lower risk of incident hip fracture in a linear trend in Icelandic community-dwelling older adults.

Introduction

Hip fractures can lead to severe health problems in older adults [1], and females are approximately three times likelier to have fractures than males [2-4]. A review of hip fracture studies shows that only half of participants regained the ability for self-care and independence as they had before fracture [1]. The risk for hip fracture differs between countries and has been reported to be higher in Scandinavia than in many other countries [5, 6]. Mortality after hip fracture is high, and studies from Spain, Sweden and Iceland showed mortality within one year after hip fracture to be 32- 43% in men and 21-30% in women [7-9].

Low mineral density (BMD) is a known predictor of hip fracture [10, 11], and BMD can be affected by genetics and lifestyle, such as physical activity and dietary intake [12]. Milk contains a number of nutrients, e.g., calcium, phosphorous, vitamins A, D and K, which can be potentially beneficial for bone health. Consequently, the Nordic Nutrition Recommendations 2012 have advocated milk and dairy consumption as part of a healthy dietary pattern as a good source of bioavailable calcium to prevent osteoporosis [13].

There is convincing evidence showing that intakes of milk and dairy are inversely related to markers of bone turnover and positively related to BMD [14, 15]. However, the associations between milk consumption and the risk of hip fracture is less certain, and two recent meta-analyses concluded that there was no clear overall association between milk intake and hip fracture risk [16, 17]. In a recent review, Malmir et al. (2020) concluded that despite an inverse association between milk and dairy intake and risk of hip fracture in cross-sectional and case-control studies, no such association was seen in cohort studies [16]. Further, a review by Hidayat et al. (2020) reported that a reduced risk of fracture with higher milk consumption was observed in prospective cohort studies from the USA (RR 0.75, 95% CI 0.65, 0.87), but not in Nordic countries (RR 1.00, 95% CI 0.85, 1.17) [17]. Adding to this

ambiguity, one Swedish study has even reported milk consumption associated with increased hip fracture risk in women [18].

Milk and dairy consumption are part of traditional Iceland dietary habits, and milk provides approximately 40% of the dietary calcium with intakes of ~300 - 400 g/day in older adults [19, 20]. The Age, Gene/Environment Susceptibility-Reykjavik (AGES-Reykjavik) Study is an Icelandic prospective cohort study with detailed lifestyle and bone health information. The present analysis aims to investigate the associations between milk intake and incidence of hip fracture in community-dwelling older adults from the AGES-Reykjavik cohort.

Methods

Study participants

The longitudinal Age, Gene/Environment Susceptibility-Reykjavik study (AGES-Reykjavik) recruited 5764 participants between September 2002 and January 2006 and followed them until 2012. Recruitment into the AGES-Reykjavik study has been described elsewhere [21]. The Icelandic National Bioethics Committee approved the AGES-Reykjavik study (VSN: 00-063), as did the National Institute on Aging Intramural Institutional Review Board (MedStar IRB for the Intramural Research Program, Baltimore, MD).

Baseline examination

The following set of measurements and information collected at recruitment were used for the present study.

Blood chemistry: During clinical examination, fasting blood samples were drawn in the first visit in the AGES study. From September 2002 to January 2006 and kept frozen at -80°C[22]. Serum 25-hydroxyvitamin D (25(OH)D) was measured using a direct, competitive

chemiluminescence immunoassay (CLIA) using the LIAISON 25(OH)D Vitamin D Total assay (DiaSorin, Inc., Stillwater, Minnesota)[23].

Body composition: Bioelectric impedance analysis (BIA) was used to measure fat and fat-free mass percentages. BMI was calculated as measured weight in kg divided by height in m squared.

Balance, muscular strength, and physical function: A computerized dynamometer chair was used to quantify the leg's extension strength with the strength of the leg measured in the right knee and quantified in terms of the maximal isometric extension force using a computerized dynamometer and dynamometer chair (Good Strength, Metitur Ltd., Finland)[24]. In this study, assessment of physical function was performed using two measurements: a) the time-up-and-go (TUG) test (the time it takes to stand up from a chair, walk 3 meters, return and sit down without using an upper limb for assistance [25], the chair used for the test was without arms [26]; b) walking 6 meters in normal speed and as fast as the participant could walk, measured in seconds and on the first try.

To assess balance, participants were asked to stand with feet separated from each other forming a V (the exact position of the V feet placement on the platform, heals apart 5 cm big toes apart 10 cm), in a relaxed upright position on a force (postural) platform, with hands beside the body [27]. While looking at a computer monitor showing pictures of five boxes in each corner and one in the middle, participants were asked to lean toward each box without moving the feet, i.e. moving their centre of mass. 15-sec breaks were taken between corners. This was done without taking an extra step or losing balance from the standing position. The greatest expanse achieved was measured for leaning to the sides from left to right and forward to backwards.

Bone measures: Quantitative Computerized Tomography (QCT) scanning was used to measure bone mineral content, volume, and density of the femoral neck. The integral variable is volumetric integral bone mineral density (vBMD, mg/cm3) of the femoral neck, reflecting both trabecular and cortical bone mass of the femoral neck region separately and quantified for the entire cohort. Procedures and quality control for these scanning measurements were described in detail elsewhere [21, 28-30].

To access lifestyle and health of participants a questionnaire was administered that included two questions, one on milk use, and another on cultured milk products with the possible answers: never, <1x/week, 1-2x/week, 3-4x/week, 5-6x/week, daily and >1x/day. Based on these two questions, a total milk estimate was calculated and categorized: < 0.5 milk servings/day, 0.5 - 0.9 milk servings/day, 1.0 - 1.4 milk servings/day, 1.5 - 1.9 milk servings/day, ≥ 2 milk servings/day. Eash serving 250 ml.

The questionnaire also included self-reported sociodemographic characteristics and health related behaviours, physical activity, and specific physician-diagnosed diseases and conditions.

Additional information on education (high vs low), marital status (yes vs no), number of medicines being taken, family history of hip fracture (yes vs no), number of falls the previous 12 months (0-1 vs > 1 fall/year), alcohol (yes vs no) smoking (yes vs no), use of calcium supplements (yes vs no), and current physical activity (yes vs no) were also collected using questionnaires.

For this study, participants missing information on the following variables were excluded: hip-fracture follow-up (n=58); those not answering questions on milk intake at baseline (n=435); and fish oil use (n=26). Participants who had previously been diagnosed with osteoporosis at baseline (n=583) or taking osteoporosis medicine without oestrogen were also

excluded (n=37). The reason for excluding those diagnosed with osteoporosis before recruitment was that those subjects are usually advised to consume milk products, which might have biased our estimates. As there was overlap of missing variables among the participants, the present analysis included 4614 subjects. Mean missing in data was 5%. Missing values of other relevant variables were imputed using SPSS "Impute missing data values".

Assessment of hip-fractures

Information on hip fractures, defined according to the ICD-10 codes S72.0, S72.1, S72.2 [31], were extracted from hospital records, an independent radiologist confirmed the fracture type [32]. The follow-up covered hip fracture occurring from enrolment in the study until the 31st of December 2012. The mean follow-up time and the standard deviation was 7.4 (SD 2.4) years.

Statistical analyses

Statistical analyses were performed using SPSS for Windows version 26.0 (SPSS, Chicago, IL, USA). In all analyses level of significance was set at $P < 0.05$ (two-sided). The normality assumption was verified by visual inspection using histograms and quantile-quantile plots. Mean, and standard deviation (SD) were used to describe continuous normally distributed variables. An independent samples t-test was used to test for differences in continuous measures between males and females. Percentages were used to describe dichotomous variables, and the chi-square test was used to compare proportions across groups.

Univariate general linear models (corrected for age and sex) were used to examine differences in continuous variables (mean, 95%CI) on possibly related to hip fracture between milk categories (< 0.5 servings/day, 0.5 - 1.9 servings/day, ≥2 servings/day).

Logistic regression models (corrected for age and sex) were used to examine whether milk in categorical variables and hip fracture risk (HR, 95%CI) were possibly related.

An additional, more complete dose-response over five different milk categories (< 0.5 milk servings/day, 0.5 - 0.9 milk servings/day, 1.0 - 1.4 milk servings/day, 1.5 - 1.9 milk servings/day, ≥ 2 milk servings/day = reference) was used to examine differences in vBMD of femoral neck (mean, 95% CI) by the univariate general linear model (corrected for age and sex). A linear trend analysis was conducted based on the categories of medians.

Finally, we examined the prospective relationship between milk intake (< 0.5 milk servings/day, 0.5 - 0.9 milk servings/day, 1.0 - 1.4 milk servings/day, 1.5 - 1.9 milk servings/day, ≥ 2 milk servings/day = reference) and incidence hip-fractures. The Cox-proportional hazard model was used starting from the time of baseline measure (September 2002 to January 2006) until hip-fracture occurred or at the end of follow-up (31st of December 2012), censuring those who died during the follow-up period. For covariate adjustment, we used three different models. Model 1 adjusted for age and sex, model 2 additionally adjusted for education, marital status, smoking, alcohol, physical activity, number of medications, TUG, balance. Model 3 was the fully adjusted model and additionally adjusted for serum 25-hydroxyvitamin D and vBMD of femoral neck. The objective of these analyses was to identify to what extent any associations between milk categories and hip-fracture risk (model 1) could be explained by baseline socioeconomics, lifestyle, health status and physical function (model 2) or baseline bone health (model 3). A linear trend analysis was conducted based on the categories of medians.

Results

In this study of 4614 participants, there were 2175 males, of whom 117 (5.4%) had a hip fracture in the follow-up. Female participants were 2439, of whom 225 (9.2%) had a hip fracture during the follow-up time. Only 1.4% of male and 2.4% of female participants reported not to drink milk or consume milk products. On the other hand, 23.1% of males and 21.4% of females consumed at least two milk servings/day, and 3.5% male and 2.3% female participants consumed ≥ 3 servings/day.

Table 1 shows the characteristics of the participants categorized by sex. Noteworthy, female participants had less muscular strength and somewhat lower physical function compared to males, and despite equally frequent intake of fish oil, females also had lower 25(OH)D than male.

Table 2 shows estimated differences (adjusted for age and gender) between milk categories in variables that are generally related to hip fracture risk. Higher milk consumption was related to higher vBMD femoral neck, higher 25(OH)D, and more frequent use of fish oil supplements.

Table 3 shows the associations between five milk categories and vBMD of femoral neck. Milk consumption was positively related to vBMD femoral neck, and a linear trend analysis based on group medians was $P < 0.001$. Categorization of this analysis by sex (**Supplemental Table 1)** shows that the relationship between milk and vBMD of femoral neck was stronger in men than in women, although the direction of the association was the same. A linear trend analysis was significant for both men and women.

Finally, **Table 4** shows the associations between baseline milk categories and incident hip fractures over a mean follow-up time of 7.4 years (SD 2.4). After adjustment for age and sex (model 1), the lowest milk category had a 59% (95% CI: 1.10-2.28) increased risk of incident

hip fracture compared to the highest category. A linear trend analysis was based on entering milk intake as a continuous variable using the median value for each intake category p-value < 0.05. Further correction for socioeconomics, lifestyle, health status and physical function (model 2) did not change the observed associations. Additional adjustment for 25(OH)D and vBMD femoral neck did attenuate the lowest milk categories HR, which was still significant but did hardly affect the HR calculated for the other milk categories. Adding in adjustment for calcium supplement did not affect the HR or significance level (data not shown). **Supplemental Table 2** shows the associations between milk intake and hip fracture categorized by sex. Whereas in the male the lower four milk categories had an around twofold risk of fracture compared to the highest category, in females only the two lowest milk categories had a 50% increased risk of fracture compared to the highest category (although not significant). Based on the further analysis in **Table 4** and **Supplementary Table 2**, there was an inverse linear relationship between milk intake and hip fracture risk for all participants. When stratifying by sex, the association between mik consumption and incident hip-fracures was more pronounced in males comapred to females.

Discussion

This study investigated the associations between milk intake and incident hip fracture in community-dwelling older adults from the AGES-Reykjavik cohort. We found that higher milk intake was associated with a lower risk of hip fracture in a linear way during ~7 years of follow-up.

Many previous cohort studies which investigated the relationship between milk intake and risk of hip fracture were done in the United States [33-36] as well as in the Nordic countries[18, 37-39], and according to a recent review on this topic [17], cohort studies from the US indicate milk consumption to be associated with lower risk of hip fracture (-25%), whereas similar studies from the Nordic countries do not. A potential explanation for this

discrepancy is that in the US, many milk products are enriched with vitamin D [40], but in the Nordic countries, vitamin D fortification of milk products is not widely implemented [41]. In Iceland, vitamin D fortification is not mandatory, but the largest local dairy company started to offer fortified milk after requests from the Icelandic Medical Directorate in 2012, which was however after the follow-up period of our study.

It is known that vitamin D promotes higher calcium absorption in the small intestines, thus resulting in greater bone mineralization. In agreement with this hypothesis, meta-regression analyses indicated vitamin D fortification as a possible effect modifier for the relationship between dairy intake and the hazards of risk fracture [17]. Furthermore, data from randomized controlled trials support the findings from cohort studies, i.e., that the combined use of vitamin D and calcium is necessary to prevent hip fracture [42].

In the present study, people in the lowest milk category (< 0.5 servings/day) had an approximately 59% higher risk of hip fracture when compared to people in the highest milk category (≥2 servings/day), and our data also show a linear relationship between milk consumption and hip fracture risk. When males and females were examined separately, the associations between milk intake and hip fracture risk were stronger in males than in females, although the direction of the association for females was in the same direction as in males. According to our data, consumption of at least two servings/day was associated with a lower fracture risk in male whereas in female consumption of at least one serving/day was associated with a lower fracture risk. Additionally, our baseline cross-sectional analyses showed that milk intake was positively related to vBMD of femoral neck, and this relationship was significant for both genders.

Traditionally, fish oil consumption has been - and still is [20] - the most important source of vitamin D in Iceland and many individuals, especially older adults, adhere to this tradition. In

the present cohort, more than 60% of both males and females reported daily fish oil intake. Interestingly, milk consumption was positively related to fish oil intake and also to approximately 6.5 nmol/L higher circulating 25(OH)D. However, statistical adjustment for 25(OH)D in the statistical model only attenuated slightly the observed associations between milk and fracture, although 25(OH)D measurement from only one-time point (baseline) was available and therefore does not provide information on 25(OH)D changes over time.

Although several epidemiological studies are available on the associations between milk and hip fracture, a publication on two large Swedish cohorts, male and female, with a very long follow-up, is of particular interest [18]. The researchers reported that milk consumption was positively related to hip fracture in female participants: there was high milk intake, i.e., 14% of female and 16% of male participants drank at least three glasses of milk a day. However, the authors also mention confounding and reverse causation as possible explanation for their findings. In comparison, less than 3% of participants drank such amounts in our study. In the Swedish female cohort, the highest milk consumption category had a 60-76% increased fracture risk (depending on the amount of statistical adjustment) compared to the low category (less than one serving/day). This study indicates that milk consumption higher than recommended [13] is not necessarily beneficial for bone health, and these adverse findings might be related to the high retinol intakes [43] and low vitamin D intakes reported in this Swedish female cohort [18].

In general, lowering the risk of hip fracture in older adults is important as the morbidity rate is high, and hip fracture is associated with low quality of life, less independence, and greater caregiver burden [44]. The mortality rate is usually high in hip fracture cases, and a recent Icelandic study showed that 36% of male and 21% of female patients died within a year from hip fracture [9]. Similar mortality in hip fracture patients was also shown in a study from Sweden (one-year mortality: 32% for males and 23% for females) [8]. In a large cohort study

conducted in Europe and the USA, mortality was increased by 2.4 for males and 1.9 for female hip fracture patients compared to their respective gender-specific participants who did not have fractures [45].

Strength and limitations

Our study has several strengths but also limitations. The FFQ used in this study did not contain any questions on cheese. Cheese provides approximately 25% of the total dietary calcium in the Icelandic diet [19, 20], and it has been indicated that cheese consumption is associated with bone health [46]. Further, only a few participants reported high milk intake >3 servings/day, thus making it impossible to detect potential negative effects of high milk consumption as indicated in another study from the Nordic countries [18].

It is a strength that our study measured important and relevant covariates, i.e., biochemical, anthropometric, functional, socioeconomic, and health-related variables. Additionally, we had information on osteoporosis at baseline and could exclude these participants from the data analysis. A further strength is that we could conduct trend analysis on the relationship between milk consumption and risk of incident hip fracture.

Conclusion

This prospective cohort study indicates that milk intake is associated with a lower risk of incident hip fracture in a linear way after ~7 years of follow-up in community-dwelling Icelandic older adults. Although milk consumption was related to fish oil consumption and higher 25(OH)D in our participants, 25(OH)D did not seem to explain the associations between milk consumption and risk of hip fracture.

Table 1: Characteristics of the participants.

Variable	Male (n=2175) Mean ± Standard deviation	Female (n=2439)	P-value*
Age (years)	76.6 ± 5.4	76.3 ± 5.7	0.094
Body composition			
Weight (kg)	83.1 ± 13.3	71.5 ± 13.1	<0.001
Height (cm)	175 ± 5	161 ± 6	<0.001
BMI (kg/m2)	26.9 ± 3.8	27.5 ± 4.8	<0.001
Fat free mass (kg)	64.0 ± 7.5	46.3 ± 6.4	<0.001
% of total body fat	21.9 ± 5.4	34.2 ± 5.1	<0.001
Integral vBMD (mg/cm3)	255 ± 50	251 ± 50	0.004
25(OH)D (nmol/L)	59.7 ± 18.0	54.8 ± 17.7	<0.001
Physical function			
Leg strength (N)	403 ± 113	260 ± 77	<0.001
Gait speed (sec)	6.5 ± 2.3	6.9 ± 2.9	<0.001
TUG (sec)	12.3 ± 3.3	12.6 ± 3.9	0.030
Lean for-back (cm)	8.9 ± 3.0	7.8 ± 3.0	<0.001
Lean left-right (cm)	11.7 ± 3.3	9.6 ± 3.4	<0.001
Count of medicines taken	4.0 ± 2.9	4.1 ± 2.8	0.258
Other characteristics			
Married (yes)	77.1%	47.9%	<0.001
Education (primary)	16.3%	28.8%	<0.001
Smoking (yes)	11.8%	12.2%	0.640
Alcohol (yes)	72.0%	59.6%	<0.001
Calcium supplements (yes)	5.8%	18.4%	<0.001
Family history of hip fracture	9.9%	10.7%	0.367
Regular physical activity[1] (yes)	34.7%	26.7%	<0.001
≥ 2 milk servings/day[2]	23.2%	21.5%	0.180
Daily fish oil	58.9%	61.9%	0.039
Hip fracture during follow-up	5.4%	9.2%	<0.001

*Independent sample t-test for a continuous variable based on a chi-squared test for categorical variables. [1]Regula physical activty as moderated or high. [2] Each serving as 250 ml.

Table 2: Estimated means of variables associated with bone health categorized by milk consumption. *

Variable	< 0.50 servings/day n= 651	0.50 - 1.99 servings/day n=2934	≥2 servings/day n=1029	P for trend*
	Mean (95% Confidence interval)			
Number of medicines	4.1 (3.9 – 4.3)	4.0 (3.9 – 4.1)	4.1 (3.9 - 4.3)	0.551
BMI (kg/m2)	27.3 (26.9 - 27.6)	27.3 (27.1 - 27.4)	27.1 (26.8 - 27.4)	0.583
Fat free mass (kg)	54.6 (54.0 - 55.2)	55.2 (54.9 - 55.4)	55.5 (55.0 – 56.0)	0.076
(%) Body fat	28.0 (27.5 – 28.5)	28.2 (28.0 – 28.4)	27.8 (27.4 – 28.1)	0.085
Bone mineral density (mg/cm3)	248 (244 – 252)	252 (250 – 254)	258 (255 – 261)	<0.001
TUG (sec)	12.5 (12.3 – 12.8)	12.4 (12.3 – 12.5)	12.6 (12.3 – 12.8)	0.402
6 m gait speed (sec)	6.7 (6.6 – 6.9)	6.6 (6.5 – 6.7)	6.7 (6.5 – 6.8)	0.375
Leg strength (N)	330 (323 – 337)	333 (330 – 337)	328 (322 – 334)	0.245
25(OH)D (nmol/L)	54.2 (52.8 – 55.6)	57.0 (56.4 – 57.7)	59.7 (58.7 – 60.8)	<0.001
Lean for-back (cm)	8.3 (8.1 – 8.6)	8.3 (8.3 – 8.5)	8.4 (8.2 – 8.6)	0.872
	OR (95% Confidence interval)			
Fish oil daily	0.42 (0.34 – 0.52)	0.68 (0.58 – 0.80)	1.00 REF	<0.001
Calcium supplements	0.95 (0.71 – 1.28)	0.88 (0.71 – 1.11)	1.00 REF	0.515

* based on general linear model (continuous variables) and logistic regression (categorical variables), both corrected for age and gender

Table 3: Differences in vBMD (B) of femoral neck (mg/cm^3, baseline) between milk categories*

Milk servings/day	participants	B (95% CI)	P-value
< 0.5	n = 595	**-9.65 (-14.66, -4.63)**	<0.001
0.5 - 0.9	n = 574	**-7.54 (-12.61, -2.47)**	0.004
1.0 - 1.4	n = 1294	**-6.51 (-10.62, -2.39)**	0.002
1.5 - 1.9	n = 799	-3.40 (-8.00, 1.21)	0.148
≥ 2	n = 909	ref.	ref.

* Based on univariate linear model adjusted for sex and age. P-value for linear trend (based on group medians) < 0.001

Table 4: Risk of hip fracture* of participants (N = 4614) categorized by milk consumption.

	milk servings/day	**participants /cases**	**HR (95% Confidence interval)**	**P-value**
Model 1	< 0.5	n = 651/55	**1.59 (1.10 – 2.28)**	0.013
	0.5 - 0.9	n = 622/54	**1.56 (1.09 – 2.25)**	0.016
	1.0 - 1.4	n = 1438/109	1.31 (0.96 – 1.79)	0.087
	1.5 - 1.9	n = 874/61	1.21 (0.85 – 1.72)	0.291
	≥ 2	n = 1029/63	1.00 (ref)	ref.
Model 2	< 0.5		**1.60 (1.11 – 2.30)**	0.012
	0.5 - 0.9		**1.56 (1.08 – 2.26)**	0.017
	1.0 - 1.4		1.34 (0.98 – 1.83)	0.069
	1.5 - 1.9		1.25 (0.87 – 1.77)	0.225
	≥ 2		1.00 (ref)	ref.
Model 3	< 0.5		**1.46 (1.01 – 2.11)**	0.045
	0.5 - 0.9		**1.48 (1.02 – 2.14)**	0.037
	1.0 - 1.4		1.30 (0.95 – 1.78)	0.102
	1.5 - 1.9		1.30 (0.91 – 1.86)	0.145
	≥ 2		1.00 (ref)	ref.

* Based on Cox regression; P-value for linear trend (based on group medians) for model 1: p = 0.004, for model 2: p = 0.004 and for model 3: p= 0.025.

Model 1: corrected for age and gender; model 2: additionally corrected for education, marital status, smoking, alcohol, physical activity, number of medications, TUG, balance; model 3: additionally corrected for 25(OH)D and vBMD of femoral neck.

Supplemental Table 1: Differences in vBMD (B) of femoral neck (mg/cm^3, baseline) between milk categories categorized by sex*.

milk servings/day	**Male (n=2175)**			**Female (n=2439)**		
	participants	**B (95% CI)**	**p-value**	**participants**	**B (95%CI)**	**p-value**
< 0.5	n = 291	-11.90 (-19.13, -4.68)	0.001	n = 360	-6.04 (-12.32, 0.24)	0.06
0.5 - 0.9	n = 273	-9.97 (-17.35, -2.60)	0.008	n = 349	-3.55 (-9.89, 2.78)	0.27
1.0 - 1.4	n = 705	-6.71 (-12.43, -0.99)	0.02	n = 733	-3.38 (-8.62, 1.86)	0.21
1.5 - 1.9	n = 402	3.39 (-9.93, 3.16)	0.31	n = 472	-1.42 (-7.24, 4.39)	0.63
≥ 2 milk	n = 504	Ref	Ref	n = 525	Ref	Ref

* Based on univariate linear model adjusted for age. P-value for linear trend (based on group medians) < 0.001 for male and p = 0.045 for female participants.

Supplemental Table 2: Risk of hip fracture* and milk consumption categorized by gender.

	Male (n=2174) cases (n=117)			Female (n=2421) cases (n=225)		
milk servings/day	**Participant/cases**	**HR (95% CI)**	**p-value**	**Participants/cases**	**HR (95%CI)**	**p-value**
< 0.5	n = 291/16	2.12 (1.05, 4.24)	0.001	n = 359/39	1.49 (0.91, 2.14)	0.12
0.5 - 0.9	n = 273/14	1.92 (0.93, 3.94)	0.07	n = 346/40	1.42 (0.93, 2.17)	0.10
1.0 - 1.4	n = 705/41	2.0 (1.12, 3.56)	0.02	n = 725/68	1.09 (0.75, 1.58)	0.66
1.5 - 1.9	n = 402/30	2.34 (1.27, 4.28)	0.006	n = 472/31	0.82 (0.52, 1.30)	0.40
≥ 2 milk	n = 503/16	Ref	Ref	n = 519/47	Ref	Ref

* Based on Cox regression adjusted for age. P-value for linear trend (based on group medians) 0.052 for male and 0.028 for female participants.

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