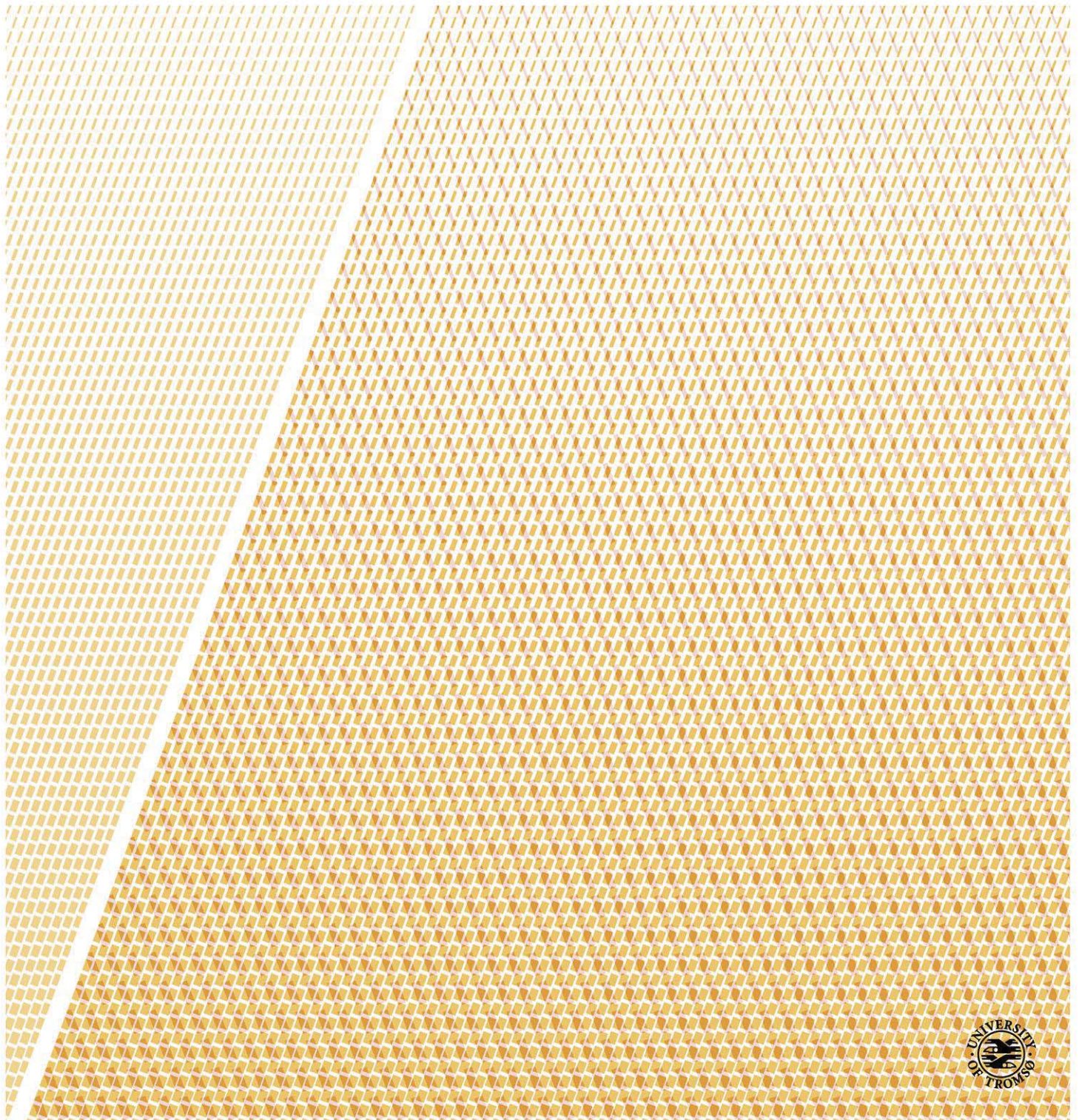Faculty of Health Sciences

Department of Clinical Medicine

# Cortical bone and fracture risk: The Tromsø Study

—

**Rita Kral**

*A dissertation for the degree of Philosophiae Doctor – November 2017*

University of Tromsø UiT The Arctic University of Norway

Faculty of Health Sciences

Department of Clinical Medicine

## Cortical bone and fracture risk: The Tromsø Study
—

**Rita Kral**
*A dissertation for the degree of Philosophiae Doctor – November 2017*

# Contents

# Acknowledgement

I would like to thank the University of Tromsø for giving me the opportunity and support to finish this study; especially the Department of Clinical Medicine and the Department of Medical Biology, UiT The Arctic University of Norway.

I also want to thank Helse Nord for believing in me and for giving me the financial support to finish this work.

I wish to express my gratitude to my principal supervisor Dr. Åshild Bjørnerem. Thank you for introducing me to the fascinating field of bone research, your scientific guidance, extensive knowledge and help throughout this work.

I also want to express my sincere gratitude to my co-supervisor Dr. Elin Richardsen, for your encouraging support and for always being available when I needed help and advice.

I am very thankful to my co-writers Marit Osima, Tove T. Borgen, Roald Vestgaard, Ingvild K. Høgestøl, Professor Ragnar M. Joakimsen and Professor Erik F. Eriksen, for their constructive comments and contribution.

Thank you, Professor Tom Wilsgaard for valuable explanations and advice.

This study would not be possible without the help from the participants of the Tromsø study and the access to the data provided by the Tromsø Study Scientific Board.

A very special thanks to the staff at the Department of Research at the University Hospital of North Norway (UNN) for recruitment of the women, staff at the Department of Radiology and Department of Radiation, UNN for scanning the patients, organized the radiation procedures and the CT images, and staff at the StraxCorp, Melbourne for analyzing the CT images.

I also want to thank the Department of Obstetrics and Gynaecology and the Department of Clinical Pathology University Hospital of North Norway.

The publication charges for Paper I have been funded by a grant from the publication fund of UiT The Arctic University of Norway.

To my good friends, thank you for your always support and love during hard times.

I am deeply thankful to my family for your endless love, support and patience.

**Summary**

**Background:** The aim of this thesis was to explore the association of the cortical architecture of the proximal femoral shaft with non-vertebral fractures. We tested the hypotheses that: (i) cortical parameters are associated with fracture risk independent of Fracture Risk Assessment Tool (FRAX) or Garvan estimates, (ii) women with fractures that are unidentified by FRAX but identified by cortical porosity have a different patient profile that contributes to their fracture risk, and (iii) women with type-2 diabetes mellitus (T2DM) have lower bone turnover markers (BTMs) and lower cortical porosity than those without diabetes, and that higher serum glucose level and body mass index (BMI) are associated with lower BTMs and cortical porosity.

**Methods:** We quantified FRAX and Garvan estimates with femoral neck areal bone mineral density (FN aBMD) and femoral subtrochanteric architecture in 211 postmenopausal women, aged 54–94 years, with non-vertebral fractures and 232 controls in a nested case-control study.

**Results:** *Paper I:* Cortical porosity and thickness were associated with fracture risk independent of FRAX and Garvan estimates. Cortical porosity but not cortical thickness improved the net reclassification of fracture cases compared with FRAX alone but not compared with Garvan.
*Paper II:* Fracture cases unidentified by FRAX but identified by cortical porosity had a patient profile different from fracture cases identified by FRAX. These patients were younger, had a higher FN aBMD, a lower FRAX score, and fewer had a prior fracture, they had higher cortical porosity, thinner cortices, and a larger total bone size than those identified by FRAX alone.
*Paper III:* Women with T2DM had a higher serum glucose, BMI, and subtrochanteric total volumetric BMD but a lower cortical porosity than nondiabetic women. Increasing serum glucose level was associated with lower BTMs and cortical porosity. Increasing BMI was associated with lower BTMs and thicker cortices.

**Conclusion:** These results suggest that cortical porosity was the most important cortical parameter associated with fracture risk. Fracture cases unidentified by high FRAX score but identified by high cortical porosity alone had a different patient profile compared with those identified by FRAX alone. Women with T2DM had lower serum levels of bone turnover markers and a lower cortical porosity than did women without diabetes. Further research is needed in larger prospective studies to determine whether cortical porosity predicts fractures independent of FRAX and can be useful in clinical practice and to examine the reasons why T2DM patients have increased risks for fracture.

**List of papers**

**Paper I**

Kral R, Osima M, Borgen TT, Vestgaard R, Richardsen E, Bjørnerem Å. Increased cortical porosity and reduced cortical thickness of the proximal femur are associated with nonvertebral fracture independent of Fracture Risk Assessment Tool and Garvan estimates in postmenopausal women. PLoS One. 2017 Sep 25;12(9):e0185363.

**Paper II**

Kral R, Osima M, Vestgaard R, Richardsen E, Bjørnerem Å. Women with fracture, unidentified by FRAX, but identified by cortical porosity, have a different patient profile that contribute to fracture risk. Submitted.

**Paper III**

Osima M, Kral R, Borgen TT, Høgestøl IK, Joakimsen RM, Eriksen EF, Bjørnerem Å. Women with type 2 diabetes mellitus have lower cortical porosity of the proximal femoral shaft using low-resolution CT than nondiabetic women, and increasing glucose is associated with reduced cortical porosity. Bone. 2017 Apr;97:252-260.

**Abbreviations**

| | |
|---|---|
| aBMD | Areal bone mineral density |
| ANOVA | Analysis of variance |
| BMI | Body mass index |
| BTM | Bone turnover markers |
| BV/TV | Bone volume as a proportion of the total volume |
| CC | Compact cortex |
| CI | Confidence interval |
| CSA | Cross-sectional area |
| CSMI | Cross-sectional moment of inertia |
| CT | Computed tomography |
| CTX | C-terminal telopeptide of type I collagen |
| CV | Coefficient of variation |
| DXA | Dual-energy X-ray absorptiometry |
| EU | European Union |
| FN | Femoral neck |
| FRAX | Fracture risk assessment tool |
| Garvan | Garvan fracture risk calculator |
| HA | Hydroxyapatite |
| HR | Hazard ratio |
| HR-pQCT | High resolution peripheral quantitative computed tomography |
| ITZ | Inner transitional zone |
| IU | International units |
| LOF | Level of fullness |
| NOK | Norwegian krone |
| NRI | Net reclassification improvement |
| OR | Odds ratio |
| OTZ | Outer transitional zone |
| PINP | N-terminal propeptide of type I procollagen |
| ROI | Region of interest |
| RR | Relative risk |
| SD | Standard deviation |
| STB | Standardized beta estimate |
| TCSA | Total cross-sectional area |
| TZ | Transitional zone |
| T1DM | Type 1 diabetes mellitus |
| T2DM | Type 2 diabetes mellitus |
| vBMD | Volumetric bone mineral density |
| WHO | World Health Organization |

# 1 Background

## 1.1 Epidemiology and burdens of fragility fracture

Fragility fracture is a growing global public health problem due to the aging population (1-4). In total, 22 million women and 5.5 million men were estimated to have osteoporosis in 2010 in the European Union (EU) (2). There were 3.5 million new fragility fractures in the EU in 2010; 18% were hip fractures, 16% forearm fractures, 15% vertebral fractures, and 51% other fractures (5). The incidence of fragility fractures shows geographic variation with a north to south gradient in Europe, with the highest incidence of hip fractures in Scandinavia (6). Norway has among the world's highest incidences of hip fractures and also a higher incidence of forearm fractures compared to other countries (7, 8). More than 9,000 hip fractures and 15,000 forearm fractures are estimated to occur in Norway annually (9-11). The age-adjusted incidence of hip fractures in women has declined by 20.4 % from 91 per 10,000 in 1999 to 74 per 10,000 in 2013 in Norway but the total number of hip fractures is expected to increase because of increasing life expectancy and because the risk of hip fracture increases exponentially after the age of 70 years (9, 12). In contrast, the incidence of forearm fractures increases exponentially in women from the age of 40 although it seems to level off after the age of 75 years (8, 13, 14).

Women experience more fractures than men and the remaining lifetime risk after the age of 50 is about 50% for women and 20% for men (15). Fragility fracture is associated with burdens to patients and society in general. Fracture patients experience pain, loss of function, disability, hospitalization, and long-term nursing care (16, 17). The increased morbidity and mortality after a fracture results in a substantial economic burden for society, including costs during hospitalization, nursing home care, and sick leave. The economic burden in the EU was estimated as €37 billion for incident and prior fragility fractures in 2010 (2). This cost is expected to increase by 25% by 2025 (2). Hip fracture is considered one of the most expensive diagnoses for the health care systems when factoring in direct and indirect costs (16, 17). In Norway, the average total costs during the first year after a hip fracture are estimated at over 500,000 NOK and the total cost of all hip fractures is calculated as 9 billion NOK in 2014 (18).

## 1.2 Risk factors of fracture

Increasing age is the most important risk factor for fragility fractures, mainly due to age-related changes in bone tissue and declines in bone mass (2). One in two women and one in five men will sustain a fragility fracture after the age of 50 (15, 19, 20). Female sex is an important risk

factor; postmenopausal women are affected more frequently and age-related bone loss is greater in women than in men (4, 21). Fragility fractures are the most common consequence of osteoporosis in both women and men (2). Low areal bone mineral density (aBMD) and low body mass index (BMI) are strong predictors of fracture (22-25). The most common non-modifiable risk factors include prior fractures, parenteral history of fracture, height, early menopause, genetic factors, and ethnicity (26-33). Furthermore, modifiable risk factors such as tobacco use, alcohol abuse, physical inactivity, propensity to fall, calcium and vitamin D deficiency, and prolonged glucocorticoid therapy are also important (26, 34-36). Several diseases are known to increase susceptibility to fractures, including rheumatoid arthritis, chronic kidney disease, type-1 (T1DM) and type-2 diabetes mellitus (T2DM), and hyper- and hypo-thyroidism (26, 37-39).

## 1.3 Fracture risk assessment

The most widely used measurement for the assessment of fracture risk is femoral neck (FN) areal bone mineral density (aBMD) measured by dual-energy x-ray absorptiometry (DXA) (40). aBMD is strongly associated with fracture (22, 26). However, aBMD has a low sensitivity for fracture (26), as most of the fractures occur in patients with aBMD in the osteopenic or normal range rather than the osteoporotic range (41, 42).

Osteoporosis is defined as "a systemic skeletal condition characterized by low bone mass and microarchitectural deterioration of bone tissue, with a consequent increase in bone fragility and susceptibility to fracture" (43). The World Health Organization (WHO) definition of osteoporosis is a aBMD 2.5 standard deviations (SD) or more below the young adult female mean (T-score ≤ -2.5 (44). Osteopenia is defined as a aBMD T-score between −1 and −2.5 SD and normal aBMD as a T-score above −1 SD (26). Fragility fracture is defined as "a fracture caused by injury that would be insufficient to fracture normal bone: the result of reduced compressive and/or torsional strength of bone. Clinically, a fragility fracture may be defined as one that occurs as a result of minimal trauma, such as a fall from a standing height or less, or no identifiable trauma" (44). Because aBMD is a low-sensitive method of identifying fractures, there is a need for new tools to improve the identification of those who are at high risk for fracture and require treatment (45). Several risk prediction models have been developed incorporating clinical risk factors and aBMD (46).

The most widely used algorithm is Fracture Risk Assessment Tool (FRAX), which calculates the 10-year probability of hip fracture and a major osteoporotic fracture (hip, clinical spine, humerus, and wrist) (47, 48). This tool includes several clinical risk factors, as shown in Table 1 (http://www.shef.ac.uk/FRAX/).

**Table 1.** Risk factors included in calculation of FRAX 10-year probability of hip and a major osteoporotic fracture* and Garvan 5-year and 10-year risk of hip and any fragility fracture**

| | FRAX | Garvan |
|---|---|---|
| **Risk factors** | | |
| | Age (40–90 years) | Age (50–96 years) |
| | Sex (female or male) | Sex (female or male) |
| | Weight (kg) | |
| | Height (cm) | |
| | Previous fracture[a] | Fractures after age of 50 years[d] |
| | Parent fractures hip | |
| | Current smoking | |
| | Glucocorticoids use[b] | |
| | Rheumatoid arthritis | |
| | Secondary osteoporosis[c] | |
| | Alcohol $\geq$ 3 units/day | |
| | Femoral neck aBMD | Femoral neck aBMD (or weight) |
| | | Falls over last 12 months[e] |
| **Type of fractures** | **A major osteoporotic fracture*** | **Any osteoporotic fracture**** |
| | Hip | Hip |
| | Clinical spine | Symptomatic spine |
| | Wrist | Wrist |
| | Humerus | Humerus |
| | | Metacarpal |
| | | Scapula |
| | | Clavicle |
| | | Distal femur |
| | | Proximal tibia |
| | | Patella |
| | | Pelvis |
| | | Sternum |

[a]A previous fracture in adult life occurring spontaneously or a fracture arising from trauma which, in a healthy individual, would not have resulted in a fracture (yes or no).
[b]If currently exposed to oral glucocorticoids or has been exposed to oral glucocorticoids for > 3 months at a prednisolone dose of $\geq$ 5 mg daily or equivalent doses of other glucocorticoids.
[c]Secondary osteoporosis (type-I diabetes mellitus, osteogenesis imperfecta [adults], untreated long-standing hyperthyroidism, hypogonadism or premature menopause (< 45 years), chronic malnutrition, malabsorption, and chronic liver disease) (http://www.shef.ac.uk/FRAX/).
[d]Number of fractures > 50 years (excluding major trauma e.g. car accidents): 0, 1, 2, or $\geq$ 3.
[e]Number of falls: 0, 1, 2, or $\geq$ 3 (http://garvan.org.au/promotions/bone-fracture-risk/calculator/)

FRAX can be used with or without FN aBMD and with trabecular bone score included (47, 49-51). Moreover, the calculation of FRAX takes into account the competing risk of death (47, 52). The FRAX algorithm has been validated in 11 prospective population-based cohorts in Europe, North America, and Asia (53), and country-specific FRAX calculators have been developed in 58 countries (50).

The Garvan Fracture Risk Calculator estimates the 5-year and 10-year absolute risk for hip and any fragility fracture (http://garvan.org.au/promotions/bone-fracture-risk/calculator/) and was developed based on data from the Dubbo Osteoporosis Epidemiology Study in Australia (54, 55). The Garvan tool includes only five risk factors: age, sex, number of fractures from the age of 50 years, number of falls over the last 12 months, and FN aBMD (or body weight) (54, 55). The Garvan Calculator can be used for individuals ≥50 years of age with or without aBMD (54, 55). It includes key risk factors for fracture and is reported to be reliable (56-59). During the development of the Garvan Calculator, the investigators tested the inclusion of other risk factors, such as corticosteroid use and family history of fracture and rheumatoid arthritis. However, they did not identify any significant association between these risk factors and fracture in their study and did not include the risk factors in the tool (54). The Garvan tool is validated in several population-based studies, including the Tromsø Study (56-58). It does not take into account the competing risk of death.

## 1.4 Bone architecture and physiology

Bone tissue has many functions. It supports soft tissue, protects vital organs, and contains the bone marrow, where blood cells are produced (60). The skeleton is important for muscle attachment and movement, serves as a storage location of minerals, and plays an important role in mineral homeostasis (61, 62). To fulfill all its functions, bone must be light to facilitate movement, hard to support the body tissue, tolerate loading and bending without breaking, and be flexible and strong to resist fracture and withstand stress (21, 63).

### 1.4.1 Composition of bone

Bone tissue is composed of a mineralized organic bone matrix and different cell types: osteoblasts, osteocytes, osteoclasts, and bone-lining cells (64, 65). The bone matrix consists of organic and inorganic components: 20–40% is organic matrix, 50–70% is mineral, 5–10% is water, and < 3% is lipids (62). The organic matrix contains collagenous proteins, mostly type I collagen (90%), and noncollagenous proteins (61). Collagen is arranged in a triple helix twinned

structure with cross-links to keep its helixes fastened (63). The inorganic bone matrix is composed mostly of hydroxyapatite (HA) $Ca_{10}(PO_4)_6(OH)_2$, which forms crystals along the collagenous fibril and also contains small amounts of carbonate, magnesium, and phosphate (66, 67). The association of HA with collagen fibers forms a composite material in which collagen provides resilience and ductility and the minerals provide stiffness and strength (61). Bone properties are influenced by the degree of cross-linking of collagen (63).

### 1.4.2 Architecture of bone: cortical and trabecular bone

The architecture of bone is optimally arranged in a structure to provide maximum strength for the least amount of bone weight (68). There are two main types of bone tissue: cortical (compact) bone and trabecular (cancellous) bone. These types are biologically identical but differ in their microstructural arrangement. Cortical bone consists of > 70% mineralized bone matrix and < 30% void volume, while trabecular bone consists of 10–30% mineralized bone matrix and 70–90% void volume (61, 66).

About 80% of the skeleton is cortical bone and forms the outer layer of the shaft of long bones and the surfaces of flat bones (66, 69, 70). Cortical bone is hard and densely packed (61). It has a slow remodeling rate because of the low surface area/volume ratio (71). Compact bone is made up of concentric lamellas surrounding a central canal containing blood vessels, nerves, and loose connective tissue that is known as the Haversian canal (Fig. 1) (60, 61). The Haversian canal, with the concentric lamellae, forms the Haversian systems or osteons, which are longitudinally oriented cylinders made up of 10–30 rings approximately 200–400 μm in diameter and 1–3 mm in length (66). The Haversian canals communicate with the marrow cavity, with the periosteum, and with each other through transverse or oblique canals called Volkmann's canals (66). These canals resemble pores on cross-sectional scanning microscopic images (71). Lacunas with osteocytes are located between and occasionally within the lamellae. Adjacent lacunae and central canals are interconnected with numerous canals called canaliculi containing cytoplasmic extensions of osteocytes (61, 72).

Trabecular bone comprises about 20% of the skeleton and is located at the metaphysis of long bones and in the vertebral bodies and flat bones (66, 70). Trabecular bone is composed of plates and rods arranged in sponge-like structures that may appear to be randomly distributed but are oriented precisely along the line of stress and weight-bearing sites (60). Within the trabecular network, there are cavities containing bone marrow. The more closely the trabecular "knots"

are located, the greater the stability and strength of the bone (61). The strengths of the trabecular bone depends on the number of trabeculae in a given volume, trabecular thickness, degree of their connectivity and the average distance between trabeculae (73).
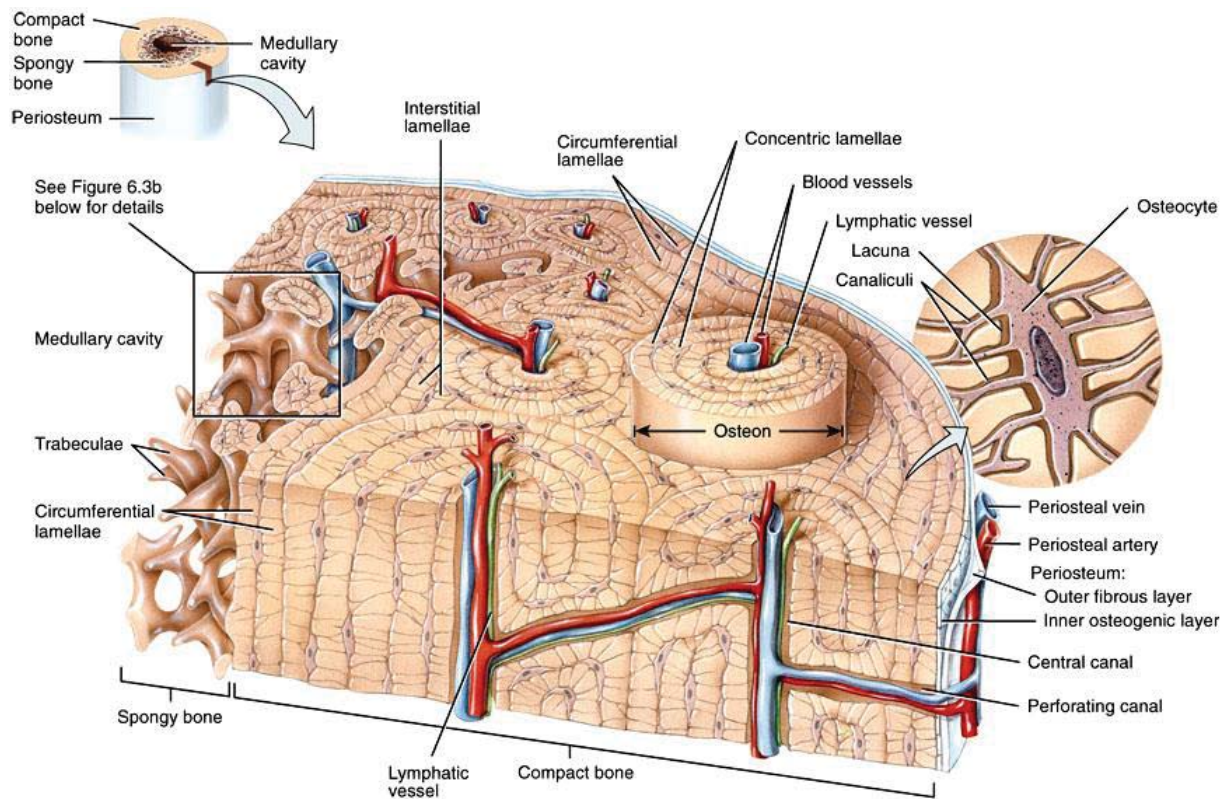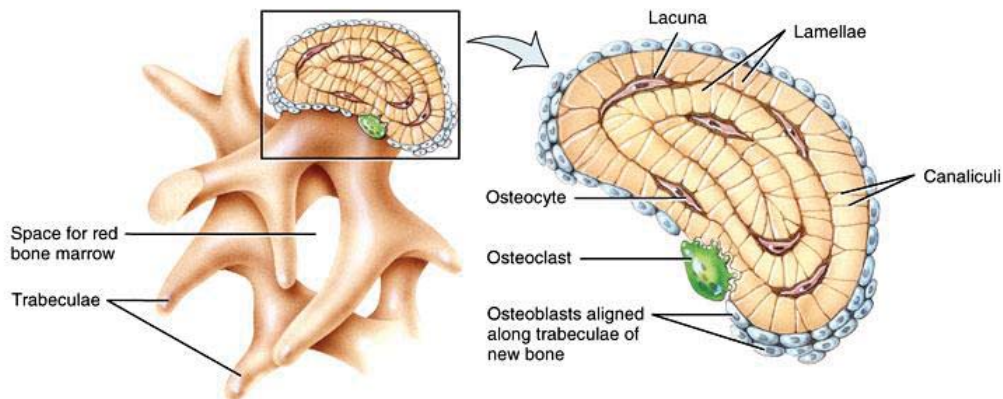
**Fig. 1.** Histology of compact and spongy bone
(a) Sections through the diaphysis of a long bone from the surrounding periosteum on the right, to compact bone in the middle, to spongy bone and the medullary cavity. The inset at the upper right shows an osteocyte in a lacuna. (b and c) Details of spongy bone. Reprinted with permission from Tortora, Derrickson, Principles of anatomy & physiology" 12th Edition (2009), Volume 1. Copyright © 2009 John Wiley & Sons, Inc. All rights reserved (60).

Trabecular bone is less dense than cortical bone and its porous structure makes it able to absorb more energy before it cracks (63). It has a larger surface area/volume ratio and higher bone remodeling rate compared to cortical bone (66, 70). The distribution of cortical and trabecular bone varies between sites and the cortical to trabecular bone ratio is 25:75 in the lumbar vertebra, 50:50 in the proximal femur, 75:25 at the distal radius (62).

### 1.4.3 Bone remodeling, repair and renewal, and physiology of bone loss

Bone remodeling is a lifelong process for the renewal of bone tissue and serves to maintain the biomechanical strength and structure of the bone (74, 75). During the bone remodeling process, damaged or old bone is broken down and removed by osteoclasts (bone resorption) and replaced by new bone formed by osteoblasts (bone formation) at the same location (74) (Fig. 2)**.** Osteoclasts are large, multinucleated giant cells that are formed by the fusion of mononuclear precursors of the hematopoietic monocyte-macrophage lineage, and their lifespan is about 1–25 days (61, 76). Osteoblasts are derived from pluripotent mesenchymal stem cells and their main function is to synthesize the organic components of the bone matrix (collagen and glycoprotein) and facilitate mineralization (64, 77). The lifespan of osteoblasts is about 1–200 days (76). Once the osteoblasts are encapsulated in the synthesized matrix, they become osteocytes (72). Osteocytes play a central role in the maintenance of the bone matrix. They are connected to each other and to the bone surface-lining cells and bone marrow cavity through an extensive network of canaliculi (65). Osteocytes have a long lifespan, which varies between 1 to 50 years (76). They coordinate the function of osteoclasts and osteoblasts in response to hormonal and mechanical stimuli (61, 74, 76). Bone-lining cells are quiescent osteoblasts that cover inactive (non-remodeling) surfaces, create a canopy over the bone remodeling compartment, and separate it from the surrounding inactive bone surfaces (78-80).

Bone remodeling is a bone surface phenomenon that occurs on the endocortical, intracortical, and trabecular surfaces and to a lesser extent on the periosteal surfaces (63, 81, 82). Bone remodeling modifies the external size and contours and internal architecture of bone by the resorption and deposition of bone matrix (61, 74, 77). Bone has the ability to respond to functional demands such as mechanical loading and to modify the internal architecture and control the shaping and replacement of bone tissue after injuries such as fractures and microdamage that occur during normal activity (74). In adults, remodeling leads to the replacement of about 10% of the skeleton every year (76, 77).
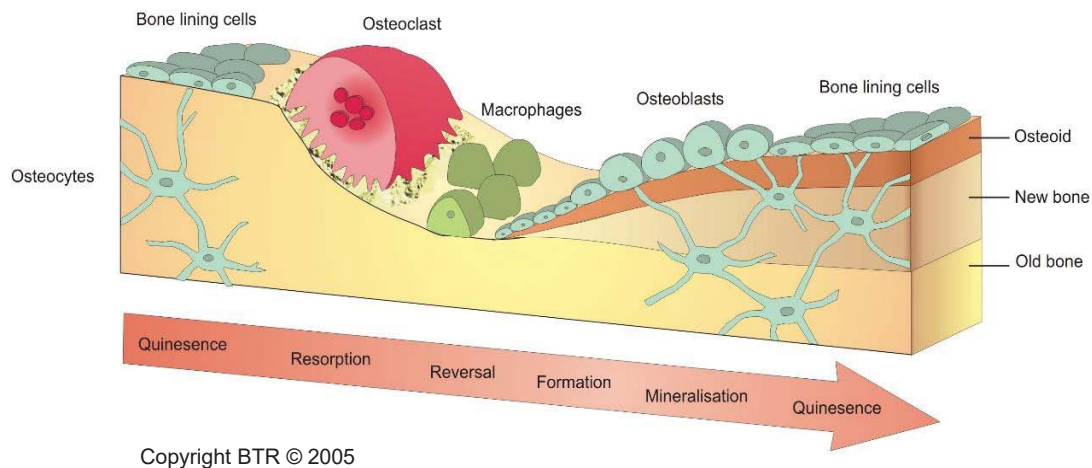
Copyright BTR © 2005

**Fig. 2.** The bone remodeling process. Bone is continuously remodeled at discrete sites in the skeleton in order to maintain the integrity of the tissue. During this process, old bone is resorbed by osteoclasts and replaced with new osteoid, secreted by osteoblasts. First, osteoclasts are activated, and the resorption phase takes approximately 10 days. Following resorption, unclassified macrophage-like cells are found at the remodeling site in the intermediate or reversal phase. Osteoblast precursors are then recruited, which proliferate and differentiate into mature osteoblasts, before secreting new bone matrix. The matrix then mineralizes to generate new bone, and this completes the remodeling process. Reprinted with permission. Copyright BTR © 2005. Biomedical Tissue Research, University of York.

In young people, there is a balance between the volume of bone removed and the volume formed (63). With advancing age, an imbalance in bone remodeling occurs and less bone is formed than resorbed, leading to bone loss (71, 76). Osteocytes play an important role in the maintenance of bone tissue, and age-related death of osteocytes is associated with the loss of bone strength before bone loss (76). Estrogen suppresses osteoclasts and, after menopause, the lower levels of estrogen result in an increased rate of bone remodeling and negative bone balance and bone loss (67).

Bone turnover markers (BTM) are enzymes and proteins released by osteoblasts during bone formation and degradation products produced by osteoclasts during bone resorption (83, 84). BTM reflect the metabolic activity of these bone cells during bone remodeling and can be monitored by serum measurement (85). Procollagen type I N-terminal propeptide (PINP) is a marker of bone formation released by the osteoblasts, while C-terminal cross-linking telopeptide of type I collagen (CTX) is a marker of bone resorption released by osteoclasts. PINP and CTX are recommended as reference markers for use in clinical studies (86). Whereas measurement of BTM are often used for monitoring osteoporosis treatment (86), its benefit in the assessment of fracture risk is not clear (87).

During age-related bone loss, increased remodeling on the trabecular surfaces results in trabecular thinning and perforation followed by the loss of trabecular plates and connectivity (61, 88). Increased remodeling on the endocortical and intracortical surfaces "trabecularizes" the cortical bone, resulting in the coalescence of pores and increased porosity due to fewer, larger pores (Fig. 3) and thinner cortices so that the cortical bone becomes emptier (with a larger proportion of void volume per unit of cortical bone volume) (63, 69). Increased cortical porosity and reduced cortical thickness reduce the resistance to crack propagation and result in the loss of compressive and bending strength (63, 89, 90). Increased periosteal apposition is suggested as an adaptive response to compensate for the loss of strength due to bone loss with ageing (63).
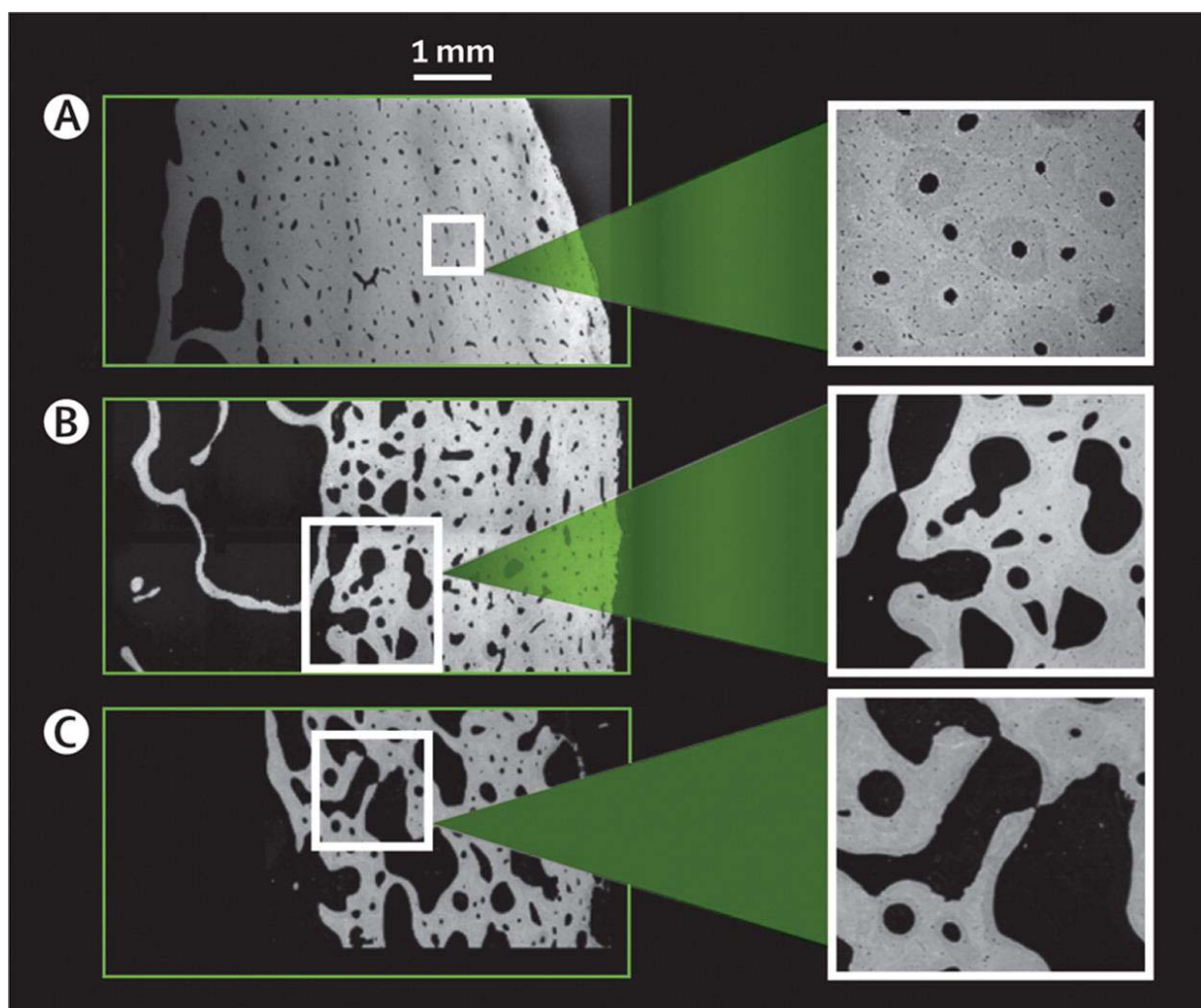


**Fig. 3**. Porosity in post-mortem specimens from three women of different ages
(A) Micrograph of a specimen from a 29-year-old woman. Pores are regular in shape and evenly distributed in the cortex. (B) Micrograph of a specimen from a 67-year-old woman. Pores are large, irregularly shaped, and have coalesced in cortex adjacent to the marrow producing cortical remnants. (C) Micrograph of a specimen from a 90-year-old woman. Most of the cortex is trabecularised by large and coalesced pores. Micrographs are of anterior subtrochanteric specimens. Reprinted from The Lancet, Zebaze et al. Copyright © 2010 with permission from Elsevier (69).

Many bone qualities others than bone mass itself contribute to skeletal fragility (63). Bone geometry (i.e. size, shape, and architecture), and bone material composition (i.e. collagen and degree of matrix mineralization) influence bone strength and fracture risk (Fig. 4) (73, 91). A larger bone diameter is an important determinant of bone strength because the resistance to bending increases to the fourth power of the bone's radius independent of bone mass in long bones (73, 92). Small increases in bone width can improve the resistance to bending and torsional loading. This is important because the highest stresses in long bones are due to bending and torsional loading (73). The cross-sectional moment of inertia (CSMI) quantitatively expresses the distribution of mass with respect to the neutral bending axis (66, 73). The deterioration of trabecular and cortical architecture compromise bone strength (93, 94) and increased cortical porosity is a potential risk factor for fracture as 80% of the skeleton is cortical bone (71).
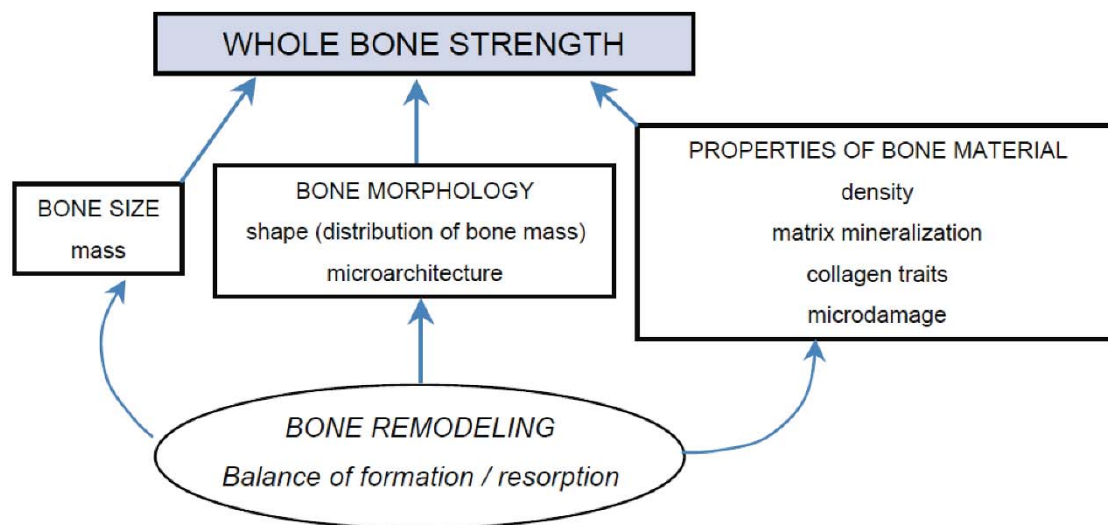


**Fig. 4.** Determinants of whole bone strength
Reprinted from Best Practice & Research Clinical Rheumatology, Bouxsein ML. Copyright [©] 2005 with permission from Elsevier (73).

# 2 Rationale and aims of the thesis

Postmenopausal women often experience fractures, resulting in pain and reduced quality of life. Hence, there is an unmet need to develop strategies for the optimal identification of individuals who are at a high risk for fracture so that they can be offered treatment. Which skeletal and non-skeletal risk factors should be included in future tools to best capture fracture risk is subject to ongoing research and discussion. FRAX and the Garvan Calculator are well-known tools that can be used with or without aBMD, which is strongly associated with fracture risk (22, 24). Using aBMD, the three-dimensional (3D) bone is assessed in a two-dimensional (2D) projection and, consequently, this method does not provide information about the 3D structural properties that makes bone fragile. The focus on trabecular bone loss in the last decades has neglected the role of cortical bone in the pathogenesis of bone fragility, although 80% of the skeleton is cortical and most bone loss is cortical (69, 71). Cortical architecture is important for bone strength but FRAX and Garvan do not consider cortical bone characteristics.

Cortical porosity is associated with fracture risk in cross-sectional studies (31, 95-98), whereas the evidence for whether cortical porosity predicts fracture is not yet clear because only a few small prospective studies have been published (99, 100). Even though the measurement of cortical porosity identified additional fracture cases and improved sensitivity for fracture compared to using FRAX or aBMD thresholds for osteoporosis (96), more than half of the women with fracture were not identified by any of these measurement methods. Therefore, improving sensitivity remains a challenge that may be met by the measurement of other structural properties or fall characteristics and the development of a new risk score.

In this thesis, we will explore the risk factors and combinations of bone morphology parameters such as cortical porosity and thickness, fall characteristics, chronic diseases, and each of the components included in the FRAX and Garvan tools to improve our understanding of the impact of these factors and their combinations on the risk of fracture. Cortical porosity is a hot topic in the field of bone research. However, the role of cortical porosity and how it is involved in the association between the above-mentioned risk factors and fractures is not well understood. Cortical porosity is reported to be associated with increased fracture risk, whereas, to the best of our knowledge, the patient profile of women who are identified by cortical porosity has not been previously published.

The availability of technology is making it possible to study the 3D architecture of bone and to identify the structural abnormalities that make bones fragile and is likely to provide a new target for the prevention of fragility fractures. This gives us a unique opportunity to examine whether the measurement of cortical porosity combined with other risk factors identifies women with high sensitivity (who sustain fractures) and specificity (who do not sustain fractures). We expect to contribute to a better understanding of the pathogenesis of fragility fractures in postmenopausal women in addition to the interplay between bone and chronic diseases such as diabetes. Defining the role of cortical porosity in bone fragility is an unmet need in this field. The role of structural decay in the cortical compartment is more and more recognized as an important contributor to bone fragility. Secondary causes of osteoporosis include T1DM, impaired kidney function, and glucocorticoids. There are few studies regarding the relationship between chronic diseases such as T2DM and trabecular and cortical bone morphology using 3D bone assessment techniques. Some studies have suggested that cortical porosity is increased in patients with T2DM (101-103). We want to achieve a better understanding of the determinants of fracture risk by exploring the association of bone architecture and chronic diseases with fracture risk.

To answer the question of why some women with fracture were identified by FRAX and others by cortical porosity, we explored whether those identified by FRAX had characteristics that differed from those identified by cortical porosity. Another interesting topic is the increased fracture risk in patients with T2DM despite preserved or increased aBMD. Increased cortical porosity has been invoked as a possible explanation for this. We therefore wanted to test whether patients with T2DM had increased cortical porosity.

**Specific hypotheses that we wanted to test:**
**i)** Measurements of cortical parameters (porosity, thickness, and area) are associated with fracture risk, independent of FRAX or Garvan estimates.
**ii)** Women with fractures who are unidentified by FRAX but identified by cortical porosity have a different patient profile that contributes to their fracture risk.
**iii)** Postmenopausal women with T2DM have lower bone turnover markers and lower cortical porosity than those without diabetes.
**iv)** Higher serum levels of glucose and BMI are associated with lower bone turnover markers and lower cortical porosity.

# 3 Materials and methods

## 3.1 Study population

The Tromsø Study is a single-center, population-based study of health issues and chronic diseases in Northern Norway, and included six surveys referred to as Tromsø 1–6 (104). The first survey was conducted in 1974, with repeated surveys conducted by the University of Tromsø in cooperation with the National Health Screening Service in 1979-80, 1986-87, 1994-95, 2001-02, and 2007-08 (104). During the Tromsø 4 survey in 1994-95, all 37,558 eligible inhabitants of Tromsø over 24 years of age were invited to participate, of which 27,158 (72%) agreed (Fig. 5). In these participants, all nonvertebral fractures that occurred between January 1, 1994 and January 1, 2010 were registered from the University Hospital of North Norway (UNN) Tromsø X-ray archives (105, 106). There is no other radiological service or fracture treatment service within 250 km of Tromsø. Therefore, the only exception would be fractures occurring while inhabitants were traveling and for which no control radiographic examination was performed after returning home. The fracture registry includes information about the time of fracture and the number and anatomical locations of all the fractures experienced by the Tromsø 4 participants (105, 106). Participants with a vertebral fracture were not included in this X-ray-based fracture registry as few of them came to the hospital for an X-ray.

In 2011, we designed a nested case-control study, which is a sub-study of the Tromsø study, and identified 1,250 women from the X-ray-based fracture registry that experienced at least one fracture of the hip, wrist, or proximal humerus after the age of 50 years (107). We invited all 760 women who still were living in Tromsø. To increase the response rate, one reminder was sent. All women who were willing to participate received a pre-screening phone call to determine whether they were eligible for participation in accordance with the inclusion and exclusion criteria (Appendix A). Those who were premenopausal women, received bisphosphonates, had pathological fractures, or had hip prostheses or metal screws in the hip region were excluded from the study. Since metal in the hip region can generate noise on computed tomography (CT) images on both sides, many women with a hip fracture could not be included unless they first had the metal removed. High energy (traffic accident) was involved in only three of 211 fracture cases and we included these cases in the study because including or excluding them did not change the results. After screening, 264 fracture cases were included in the study.
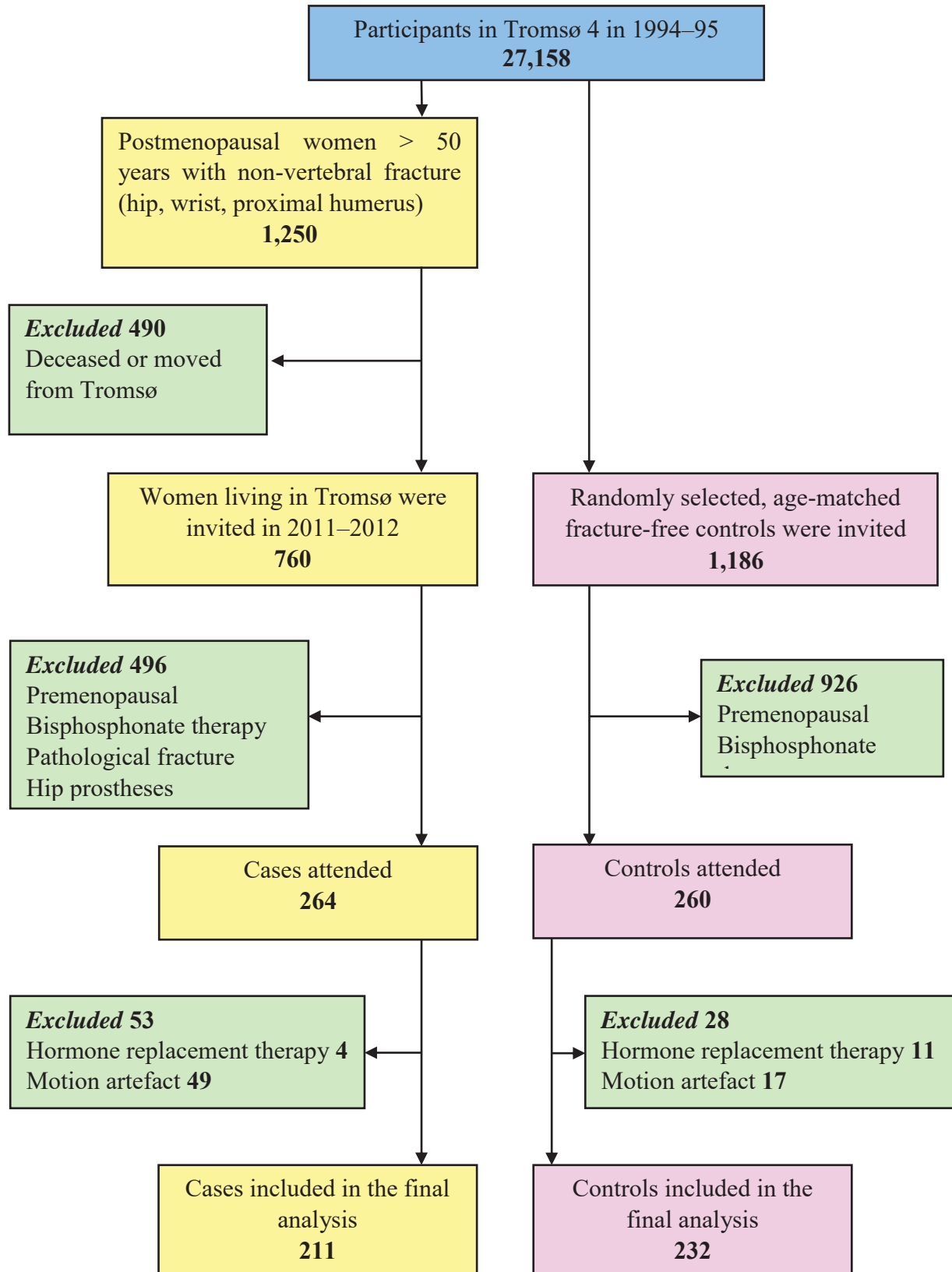
**Fig. 5.** Participants in this nested case-control study based on the Tromsø Study in 1994–95

Age-matched, fracture-free women who were within the same 5-year age groups were randomly selected from among the Tromsø 4 participants and 1,186 were invited. After a pre-screening phone call to determine whether they were eligible and fracture-free, 260 controls were included. Of these 524 participants, we excluded 15 women who were currently receiving hormone replacement therapy and 66 women owing to motion artefacts during CT scans. Motion artefacts occur with voluntary or involuntary patient movement during image acquisition and appear as blurring, streaking, or shading on the CT image and degrade image quality (108). Of these 66 women who were excluded due to motion artefacts, 49 were fracture cases and 17 were controls. The 49 excluded cases with motion artefacts were 3.2 years older than the 215 cases without motion artefacts ($71.6 \pm 1.2$ vs. $68.3 \pm 0.5$), ($p = 0.010$). The 17 excluded controls with motion artefacts were non-significantly one year older than the 246 controls without motion artefacts ($69.2 \pm 2.2$ vs. $68.2 \pm 0.4$), ($p = 0.569$). This resulted in 443 women in the final analyses: 232 controls and 211 fracture cases (four hip, 181 wrist, and 26 proximal humerus). The median time since their index fracture was 6.6 years (range, 1–25).

## 3.2 Ethics

The study was approved by the Regional Committee for Medical and Health Research Ethics (REK Sør-Øst reference 2010/2282) and was conducted in accordance with the World Medical Association Declaration of Helsinki. All participants provided written informed consent.

## 3.3 Data from questionnaires and measurements

At enrollment, the participants completed a self-administered questionnaire that included information concerning all fractures occurring after the age of 50 years (number and type of fracture), number of falls in the last year, diseases, use of medication, and lifestyle factors such as exercise and smoking (Appendix B). Hours of exercise per week were calculated as weekly exercise frequencies multiplied by hours per session. The self-reported diagnosis and duration of T2DM was confirmed based on information in medical records, and none of the participants had T1DM. Diabetic complications were also identified through the medical records.

Height and weight were measured while wearing light clothing and no shoes. BMI was calculated as weight/height². Total hip and FN aBMD were measured at the non-dominant side using dual-energy X-ray absorptiometry (DXA, GE Lunar Prodigy, Lunar Corporation, Madison, WI, USA) and the coefficients of variation (CV) were 1.2% and 1.7%, respectively. In women with a hip fracture on the non-dominant side, the opposite dominant side was used.

Fasting blood samples were collected between 8 and 10 a.m. and assayed at the University Hospital North Norway for serum glucose (Roche Diagnostics, Germany with a CV of 0.5–1.6%); for insulin (Elecsys 2010 Modular Analytics E170, Roche Diagnostics, Germany, with a CV of 0.8–4.6%); at Haukeland University Hospital, Bergen for 25-hydroxyvitamin D (25[OH]D) (mass spectrometry, with a CV of 4.0–4.6%); for parathyroid hormone (PTH) (Immulite 2000, with a CV of 7–12%); for creatinine, measured photometrically with a CV of 3%; and at the Hormone Laboratory of Oslo University Hospital Aker for PINP and CTX using electrochemiluminescence immunoassays (Elecsys 1010 Analytics, Roche Diagnostics, Germany with a CV of 3–8%).

Homeostatic model assessment of Insulin Resistance (HOMA-IR) was calculated using the following formula: (glucose multiplied by insulin)/135 (109). Kidney function was assessed using estimated glomerular filtration rate, which was calculated using the Chronic Kidney Disease Epidemiology Collaboration equation (110).

### 3.4 FRAX and Garvan estimates

We entered data collected at enrollment into the online country-specific FRAX algorithm for Norway to calculate the individual 10-year probability of a major osteoporotic fracture (http://www.shef.ac.uk/FRAX/) and the Garvan Fracture Risk Calculator to calculate the 10-year fracture risk for any fragility fracture (http://garvan.org.au/promotions/bone-fracture-risk/calculator/). An age of 90 years was used to obtain FRAX estimates in individuals older than 90 years of age. We included FN aBMD in the calculation of FRAX and Garvan estimates. When we included secondary causes of osteoporosis such as diabetes, hyperthyroidism, early menopause (< 45 years of age), and malabsorption in the calculation of FRAX estimates, which are well-known risk factors for fracture, all FRAX estimates remained completely unchanged. Whether these risk factors are independent of aBMD is uncertain and it is assumed that the fracture risk is mediated by aBMD (47). Therefore, when aBMD is included in the FRAX estimate, no further weight is accorded by the inclusion of secondary causes of osteoporosis (47). The index fractures used as the inclusion criteria for this study were not included as "previous" fractures in the calculation of FRAX estimates because the aim was to assess the 10-year probability of fracture before the event rather than the probability of fracture after this event. The index fractures were not included in the number of fractures in the Garvan estimate.

However, "previous fracture" (before the index fracture) and "subsequent fracture" (after the index fracture) should both be used equally in the calculation of FRAX and Garvan estimates. During the calculation of Garvan estimates, we identified an inconsistency between the self-reported total numbers of fractures and self-reported "previous fractures," as shown in Table 2. For example, 20 women reported that they had experienced only one fracture despite reporting a previous fracture (before the index fracture), and 37 women reported that they had experienced two or more fractures despite reporting no previous fracture. Therefore, we decided to validate fracture events in 91 women through medical records in those who either had two or more self-reported fractures (n = 71), a previous fracture (n = 54), or both (n = 34). We did not validate the fractures in 120 other fracture cases because of the consistency between the self-reported total numbers of only one fracture and no previous fracture.

**Table 2.** Self-reported numbers of fractures vs. previous fractures based on questionnaires

|  | Numbers of fractures including index fracture | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
|  | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | Total |
| Yes, fracture before index fracture | 20* | 21* | 9* | 1* | 1* | 0 | 2* | 0 | 54 |
| No, fracture before index fracture | 120** | 29* | 3* | 2* | 0 | 0 | 2* | 1* | 157 |
| Total | 140 | 50 | 12 | 3 | 1 | 0 | 4 | 1 | 221 |

*Fractures in these 91 cases were validated through medical records
**Fractures in these 120 cases were not validated through medical records

The agreement between self-reported previous/subsequent fractures vs. those identified through medical records is shown in Table 3. Of 54 women who reported that they had experienced a previous fracture, 33 were confirmed and 28 additional fracture cases were identified through medical records. Thus, a total of 61 women had a validated previous or subsequent fracture.

**Table 3.** Agreement between previous/subsequent fractures
based on self-reported questionnaires vs. medical records

| Self-reported | Medical records | | |
|---|---|---|---|
|  | No | Yes | Total |
| No | 9 | 28 | 37 |
| Yes | 21 | 33 | 54 |
| Total | 30 | 61 | 91 |

The agreement between self-reported numbers of fractures vs. those identified through medical records is shown in Table 4. Some of the reasons for misclassification of fracture cases were: i) writing errors in the questionnaire, ii) fractures of fingers and toes being included as a previous fracture before but not after the validation, and iii) limited information being available

from electronic medical records before 1995, leading to fractures that occurred more than 22 years ago being missed. The types of fractures included as previous/subsequent fractures after the validation were fractures of the wrist, humerus, patella, elbow, proximal tibia, foot, ankle, pelvis, rib, spine, and clavicula.

**Table 4.** Agreement between the self-reported numbers of fractures through questionnaires vs. those identified through medical records

| Self-reported | Based on medical records | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | Total |
| 1 | 14 | 3 | 3 | 0 | 0 | 0 | 20 |
| 2 | 11 | 33 | 6 | 0 | 0 | 0 | 50 |
| 3 | 3 | 5 | 4 | 0 | 0 | 0 | 12 |
| 4 | 0 | 2 | 1 | 0 | 0 | 0 | 3 |
| 5 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 7 | 2 | 1 | 0 | 0 | 0 | 1 | 4 |
| 8 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| Total | 30 | 44 | 15 | 1 | 0 | 1 | 91 |

This validation process identified 61 women with previous or subsequent fractures through medical records. Each of these 61 women had a total of two or more fractures (**Table 5**). We included this information in the latest calculation of their FRAX and Garvan estimates. The updated FRAX estimates changed only modestly for those women who were reclassified, either because they had a previous fracture reported that was not confirmed (n = 21) or no self-reported previous fracture identified through the questionnaires but a previous or subsequent fracture identified through the medical records (n = 28), as shown in Table 3.

**Table 5.** Numbers of fracture vs. previous/subsequent fracture through medical records

| | Total number of fracture including index fracture | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | Total |
| Previous/subsequent fracture | 0 | 44 | 15 | 1 | 0 | 1 | 61 |
| No previous/subsequent fracture | 30 | 0 | 0 | 0 | 0 | 0 | 30 |
| Total | 30 | 44 | 15 | 1 | 0 | 1 | 91 |

## 3.5 Quantification and validation of bone architecture

CT scans (Siemens Somatom Sensation 16, Erlangen, Germany) of the non-dominant hip were performed at the Department of Radiology at the University Hospital of North Norway (96). The CT machine had an in-plane resolution of 0.74 mm, and the slice thickness was set at 0.6 mm. The hip was scanned from just above the femoral head to 2 cm below the lesser trochanter and the exposure dose of radiation was ~1.5 mSv (Fig. 6). CT scans of the hip were performed at 120 kV with a pitch of 0.75 using 90 mA and reconstructed using a fixed field of view at 120 mm. Quality control was performed by scanning a phantom containing rods of hydroxy-apatite (QRM Quality Assurance in Radiology and Medicine GmbH, Moehrendorf, Germany).
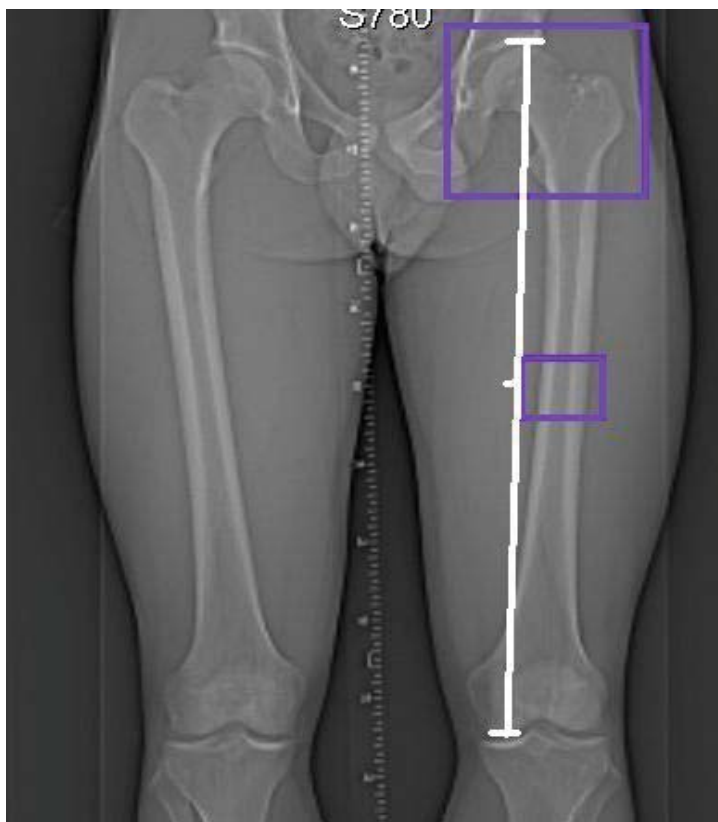


Topogram (Scout) 768 mm:
The scan must include the knee joint, the entire hip joint, and the acetabulum using the "CaudoCranial" scan direction.

Scan 1 - Hip:
The scan must include the acetabulum and 2 cm below the lesser trochanter. Use a fixed field of view (FOV) at 120 mm. FOV should only be increased if the entire femoral neck is not included in the FOV.

Scan 2 - Femur Midshaft:
Use the measuring tool and locate the middle part of the femur.
Scan length: 5 cm
Small FOV: 50 mm

**Fig. 6.** Computed tomography protocol of the proximal femur and femur midshaft

**Image reconstructions: Hip\Knee:** One plane; only axial slices.
Scan 2 box must be reconstructed with the FOV in the middle of the bone marrow cavity.

|  | Slice thickness/increment | Algorithm/kernel | Window/Level |
|---|---|---|---|
| Scan 1 | 0.6/0.6 | B30s medium | Bone 3000\150 |
| Scan 2 | 0.6/0.6 | B30s medium | Bone 3000\150 |

**Scan parameters:**

|  | Patient position | Spiral | kV | Ref. mAs | Rotation time | Slice collimation | Pitch |
|---|---|---|---|---|---|---|---|
| Scan 1 | Head first supine | Spiral | 120 | 90 | 1s | 40 × 0.6 mm | 0.75 |
| Scan 2 | Head first supine | Spiral | 100 | 150 | 1s | 40 × 0.6 mm | 0.75 |

The CT images were sent to Melbourne, Australia, and analyzed by collaborators who were blinded to the fracture status and diabetes status using the StrAx1.0 software (StraxCorp Pty Ltd, Melbourne, Australia) (111). As cortices are thin at the most proximal femur (femoral head, neck, and trochanter), analyses were confined to a 3.7 mm subtrochanteric region of interest (ROI) with thicker cortices, which started at the tip of the lesser trochanter (Fig. 7).
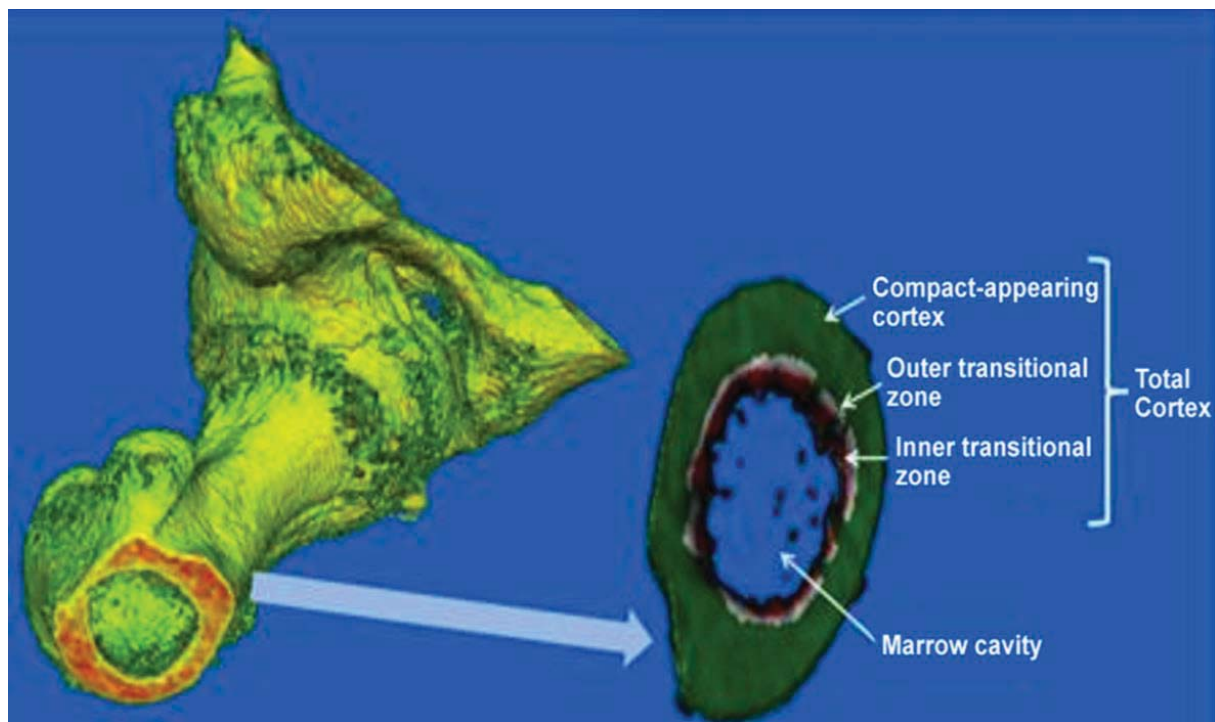


**Fig. 7.** Cross-section image of proximal femur and its compartments
Segmented computed tomography image obtained at the proximal femur using StrAx1.0, a non-threshold-based segmentation algorithm, showing the total cortex (the area used for the cortical porosity measurements), consisting of the three cortical compartments: compact-appearing cortex, outer and inner (red) transitional zones, and trabecular bone area. Porosity was assessed from QCT slices distal to the lesser trochanter. Reprinted from Journal of Bone and Mineral Research, Zebaze et al. Copyright$^©$ 2016, with permission from John Wiley and Sons (112).

The subtrochanteric region within the ROI in CT images was segmented into the compact-appearing cortex, transitional zones (TZ), and trabecular compartment using StrAx1.0. This is a non-thresholding method that automatically selects attenuation profile curves similarly in low-resolution images at the subtrochanteric site (96, 112) as in HR-pQCT images at distal radius and distal tibia (111). Local bone edges were identified at the beginning and end of the rising and falling S-shaped portions of the curve, which enabled the delineation of the compartments (Fig. 8) (111). Of the total cortex at the subtrochanteric site, 70.0% was compact-appearing cortex, while 22.3% and 11.7% were outer (OTZ) and inner transitional zone (ITZ), respectively.

Bone was segmented by analyzing ~3,600 consecutive overlapping profiles around the perimeter of each cross-sectional slice. The density profile curve produced had two plateaus: one corresponding to the compact-appearing cortex and one corresponding to the trabecular compartment. Between these plateaus was a descending S-shaped curve or transition. This is the TZ. The density profile curve expressed the mineralized bone area as the percentage of total area within each column (111).
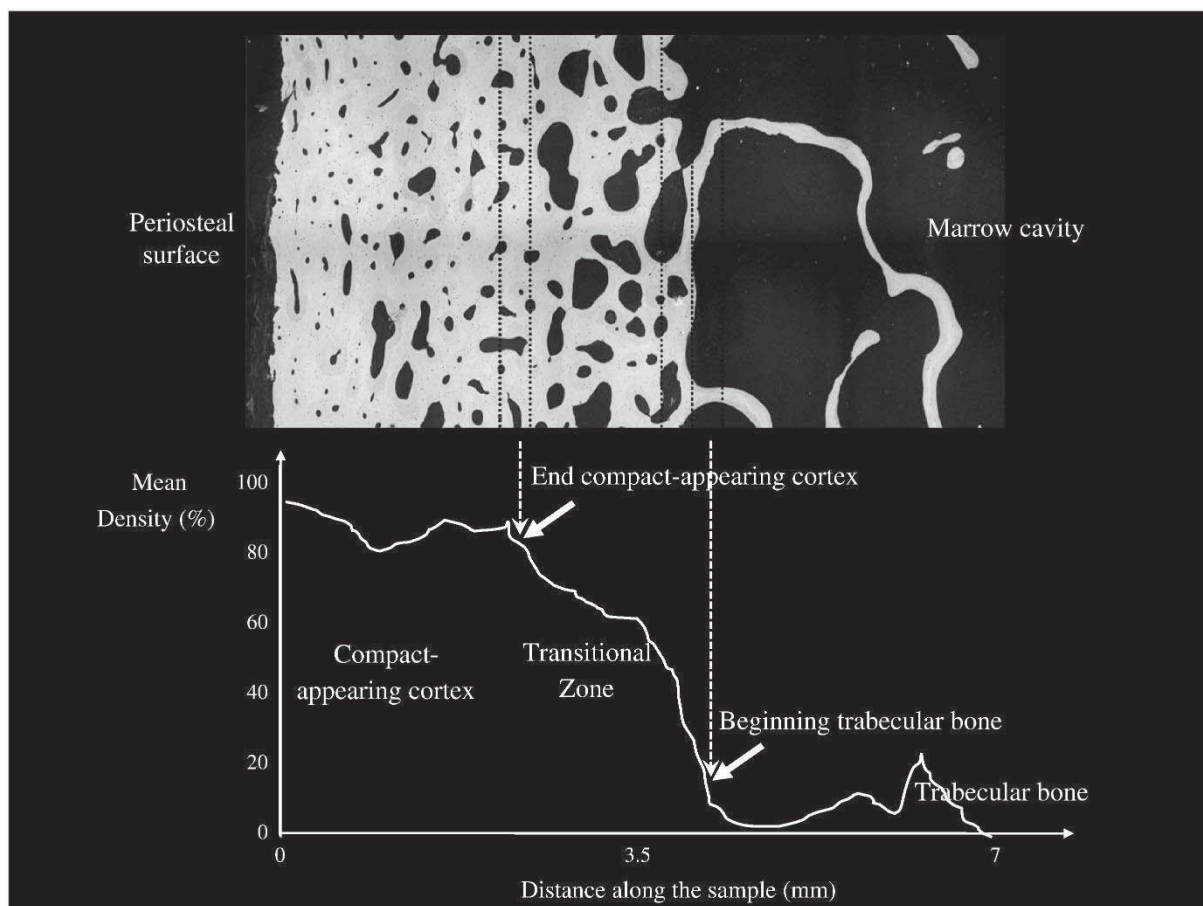


**Fig. 8.** A scanning electron microscopic image of the subtrochanteric showing the compact-appearing cortex, transitional zone, and trabecular compartment. The density profile curve produced has two plateaus; one corresponding to the compact-appearing cortex and one corresponding to the trabecular compartment. Between these plateaus is a descending S shaped curve or transition between the two plateaus. This is the transitional zone. The y-axis is the density profile curve expressing the mineralized bone area as the percentage of total area within each column (black dotted rectangles). Reprinted from Bone, Zebaze et al., Copyright © 2013 with permission from Elsevier (111).

Cortical porosity in the total cortex was quantified automatically throughout the ROI similarly in CT images as in HR-pQCT images even though pores were not visible (111). Porosity values presented here are the mean proportion of emptiness within each voxel or the fraction of the bone volume occupied by void regardless of the size of the pores. This is a density-based,

indirect measure of porosity, and the size and number of pores were not determined (96, 111, 112).

To measure porosity at the sub-voxel level, two referent attenuation values are required: P, the background (muscle, water etc.) and B, the fully mineralized bone matrix (1200 mg HA/cm³). The proportion of the voxel volume occupied by mineralized bone matrix volume is its level of fullness (LOF). As previously reported, the LOF of each voxel is estimated as (LOF) % = (Ai-P)/(B-P), where Ai is the attenuation of voxel *I* (96). From the LOF, the void volume of each voxel or level of emptiness (porosity) = 100 – LOF (%) (Fig. 9) (96).



**Fig. 9.** To measure porosity, two referent attenuation values are required P: the background (muscle, water etc.) and B: the fully mineralized bone matrix (1200 mg HA/cm³). The proportion of the voxel volume occupied by mineralized bone matrix is its level of fullness (LOF) and is estimated as (LOF) %=(Ai-P)/(B-P), where Ai is the attenuation of voxel i. From LOF, the void volume o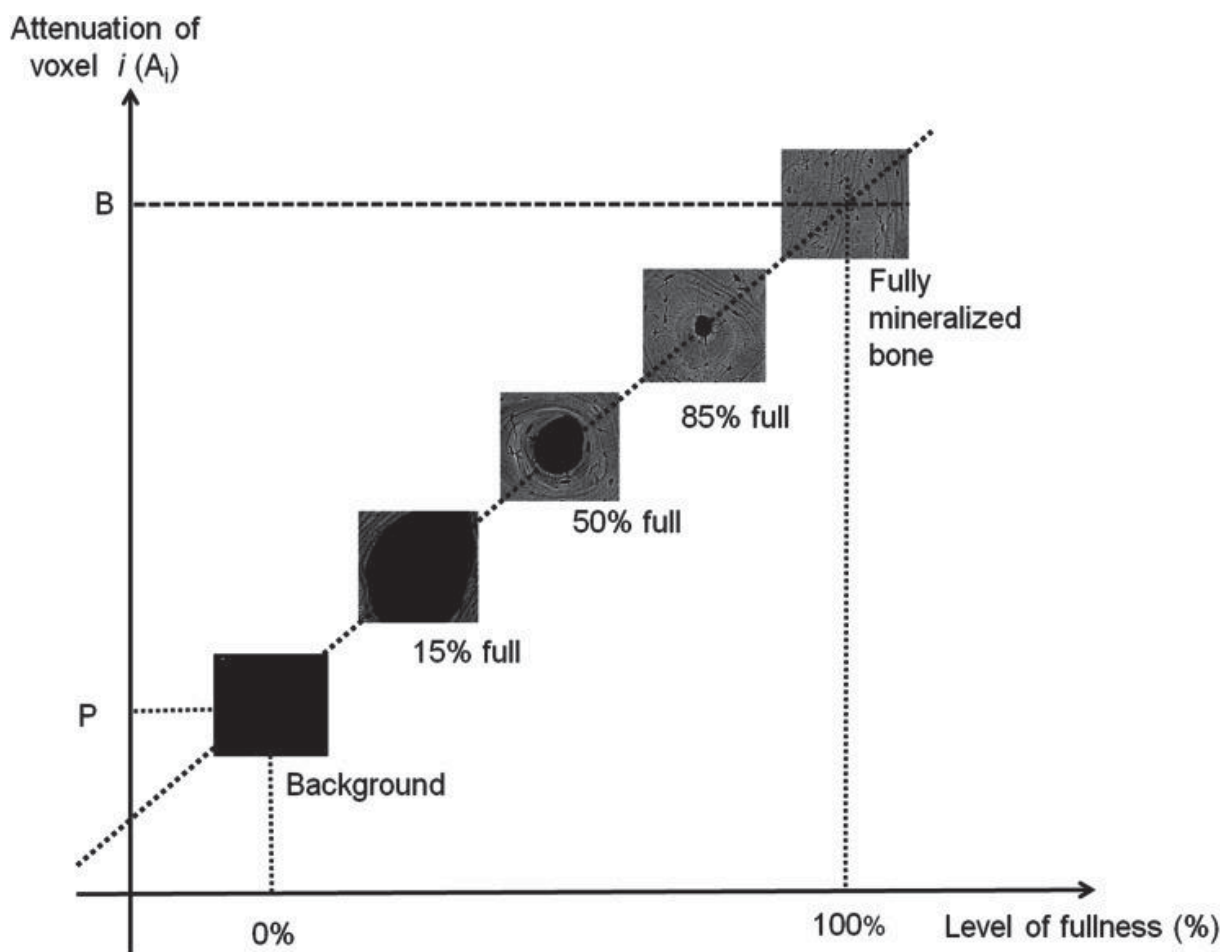f each voxel or level of emptiness (porosity)=100–LOF (%). Reprinted from Osteoporosis International, Ahmed et al., Copyright © 2015 with permission from Springer (96).

StrAx1.0 accounts for the partial volume effect by including not only void within the completely empty voxels but also the partly empty voxels. By using the StrAx1.0 software, we quantified porosity of the compact cortex and the TZ. It was thus more inclusive than traditional measurements and the values for porosity were higher than those reported using other methods.

The accuracy of porosity measurements using CT with a voxel size of 740 µm was validated *ex vivo* by testing the agreement with HR-pQCT measurements with a voxel size of 82 µm of the same ROI at the femoral subtrochanter in cadaveric specimens (96). The agreement ($R^2$) between CT and HR-pQCT ranged from 0.86 to 0.96 for the quantification of porosity at the same femoral subtrochanteric site (range 40–95%) (96). As shown in the Bland-Altman plots, the error (difference between measurements by CT and HR-pQCT scanning) ranged from 0% to 10% depending on the compartment and agreement between both measurements exceeded 90% (96, 112). For ethical reasons, it was not possible to perform *in vivo* validation by rescanning women on the same day. Additional validation of the StrAx1.0 software analyses of the femoral subtrochanter cortical porosity as well as all standard CT parameters in this current study was performed by repositioning and rescanning a human hip phantom (consisting of a human pelvic skeleton embedded in plastic material) 10 times, with the CV between 0.3% and 2.3% (96). This human hip phantom was delivered with the CT scanner (Siemens Somatom Sensation 16, Erlangen, Germany).

We presented the following variables in our study: femoral subtrochanteric porosity of the total cortex, compact appearing cortex, and the OTZ and ITZ zones; total and cortical volumetric BMD (vBMD); trabecular bone volume/tissue volume ratio (BV/TV); the total, medullary, and cortical cross-sectional area (CSA); and cortical thickness. In addition, we used the cortical CSA/total CSA ratio as a measurement of relative cortical thickness because cortical thickness varies around the perimeter of the bones. A smaller cortical area relative to the total area reflects greater excavation on the endocortical surface relative to periosteal apposition, which enlarges the medullary canal area while producing a smaller cortical area and thus a thinner cortices relative to the total area (31). We also used bone strength estimates such as CSMI, which were all quantified by the StrAx1.0 software.

## 3.6 Statistical analysis

When we designed this study, we used EpiInfo (version 2008) for power calculation to assess the number of participants needed. With cortical porosity as a continuous variable, we chose a threshold to define those who were exposed. Assuming a power of 80% and a significance level of 5%, we would be able to detect an odds ratio (OR) of 2.0 with 165 fracture cases and 165 controls (1:1), OR of 1.8 with 230 cases and 230 controls (1:1), and OR of 1.6 with 363 cases and 363 controls (1:1) if 25% of the sample was exposed to high porosity (Paper I).

Age-adjusted analysis of variance (ANOVA) was used to compare differences between cases and controls (Paper I). Logistic regression analysis was used to calculate the OR for fracture with a 95% confidence interval (CI) adjusted for age, height, and weight, and additionally adjusted for Garvan and FRAX estimates and expressed per one SD difference in FN aBMD, FRAX, and Garvan estimates and cortical parameters. Due to the skewed distribution of FRAX and Garvan estimates, we used log-transformed variables in the models. To further discriminate fracture cases from controls, the area under the receiver operating characteristic curve (AUC) was obtained using logistic regression models for FRAX and Garvan estimates alone and after adding cortical parameters (porosity, thickness, or area). Sensitivity and specificity for fracture were explored at selected thresholds for FRAX estimates above 15%, 20%, and 25%, Garvan estimates above 15%, 20%, and 25%, cortical porosity above the 75th, 80th, and 90th percentile, and cortical thickness below the 10th, 20th, and 25th percentile. We chose specificity above 85% as a reasonable criterion for the selection of thresholds for each of the variables and for further analysis of combinations of variables. We calculated the net reclassification improvement (NRI) to quantify how well the new models correctly reclassified the women (Table 6) (113).

**Table 6.** Formula and interpretation of net reclassification improvement (NRI)

| Event NRI | Pr(up\|event) – Pr(down\|event) = (number of events classified up – number of events classified down)/number of events <br> The net percentage of persons with the event correctly classified upward |
|---|---|
| Nonevent NRI | Pr(down\|nonevent) – Pr(up\|nonevent) = (number of nonevents classified down – number of nonevents classified up)/number of nonevents <br> The net percentage of persons without the event correctly classified downward |
| Overall NRI | [Pr(up\|event) – Pr(down\|event)] + [Pr(down\|nonevent) – Pr(up\|nonevent)] = event NRI + nonevent NRI <br> The sum of the net percentages of correctly reclassified persons with and without the event of interest; this statistic is implicitly weighted for the event rate and cannot be interpreted as a percentage |

Pr = probability. (This table is a modified version of the table from Leening et al.) (113).

Each of the original models with FRAX alone or Garvan alone was compared with a new model, which was the original model plus cortical porosity or cortical thickness. The overall NRI was the sum of correctly reclassified women with fracture (event) and without fracture (nonevent).

In Paper II, we presented mean and standard error of the mean (SE) in four groups. Group 1: 35 women with fracture identified by high FRAX score (threshold >20%) but unidentified by high cortical porosity (threshold > 80th percentile). Group 2: 43 women with fracture unidentified by high FRAX score but identified by high cortical porosity. Group 3: 115 women with fracture unidentified by both high FRAX score and high cortical porosity. Group 4: 232 age-matched fracture-free controls. The characteristics of the four groups were compared using age-adjusted ANOVA and the bone traits were compared after additional adjustment for height and weight.

In Paper III, we used the same data as used in Paper I and Paper II and the data from fracture cases and controls in the nested case-control study were pooled and analyzed as data from a cross-sectional study. Normally distributed continuous variables are presented as the means ± SD. The remaining variables (trabecular BV/TV, serum insulin, and HOMA-IR) are presented as medians (range). To correct for a skewed distribution, we used log-transformed trabecular BV/TV in the analysis. Differences between women with and those without T2DM were assessed using analysis of covariance (ANCOVA) adjusted for age and fracture status. The results are presented stratified by T2DM-status and fracture status. In sub-analysis, we compared diabetic women with and without fracture and nondiabetic women with and without fracture using ANCOVA adjusted for age and BMI. Scatterplots of PINP, CTX, cortical porosity, and cortical thickness as a function of serum glucose and BMI are presented. Linear regression analysis was used for associations of BTM and bone architecture (y) as a function of glucose and BMI (x) adjusted for age and fracture status. Standardized regression coefficients (standardized beta estimates) were used to facilitate the comparison of the strength of associations between the exposure and endpoints.

Analyses were performed using STATA Software, v14 (StataCorp, LP, Tx, USA), and SAS software, v9.3 and v9.4 (SAS Institute Inc., Cary, NC, USA). All tests were two-sided and a p-value < 0.05 was considered statistically significant.

# 4 Main results

## 4.1 Paper I. Cortical parameters, FRAX, Garvan estimates, and fracture risk

FRAX and the Garvan Calculator are widely used to assess fracture risk. However, these tools do not include measurements of cortical architecture, which may provide independent information beyond that provided by these conventional approaches. We tested the hypothesis that measurements of cortical parameters (porosity, thickness, and area) are associated with fracture risk independent of FRAX or Garvan estimates (114).

This nested case-control study included 211 postmenopausal women, aged 54–94 years, with nonvertebral fractures and 232 controls from the Tromsø Study, Norway (Table 7). We assessed FRAX and Garvan 10-year risk estimates for fragility fractures and quantified femoral subtrochanteric cortical porosity, thickness, and area on CT images using StrAx1.0 software.

Increased cortical porosity and reduced cortical thickness but not smaller cortical area remained associated with fracture independent of FRAX and Garvan estimates. Adding cortical porosity and thickness to FRAX increased the AUC. A measurement of cortical porosity (> 80th percentile) or cortical thickness (< 20th percentile) identified 20.4% and 17.5% additional fracture cases that were unidentified using FRAX alone and 16.6% and 13.7% fracture cases unidentified using Garvan alone (114). Cortical porosity but not cortical thickness improved the net reclassification of fracture cases compared with FRAX alone but not compared with Garvan alone. In conclusion, cortical parameters may help improve the identification of women at risk for fracture.

## 4.2 Paper II. Patient profiles in those identified by cortical porosity but not by FRAX

Cortical porosity is associated with the risk for fracture independent of FRAX. We wanted to test the hypothesis that women with fracture who are unidentified by FRAX but identified by cortical porosity have a different patient profile that contributes to their fracture risk.

We quantified FRAX scores with FN aBMD included and femoral subtrochanteric architecture in 211 postmenopausal women aged 54–94 years with nonvertebral fractures and 232 controls in Tromsø, Norway.

The 43 fracture cases unidentified by FRAX but identified by porosity > 80th percentile were younger, had higher FN aBMD and, fewer had a prior fracture, they had higher cortical porosity, thinner cortices, larger total and medullary CSA, higher CSMI and lower cortical and total vBMD than 35 fracture cases who were identified by high FRAX score but not by high porosity. Fracture cases unidentified by FRAX but identified by cortical porosity had a patient profile, which captured additional fracture risk components not captured by FRAX.

**4.3 Paper III. Type 2 diabetes mellitus, cortical porosity, serum glucose, and BMI**
Increased cortical porosity is invoked to be associated with increasing fracture propensity in patients with T2DM. This is a paradox because increased cortical porosity is generally associated with high bone turnover, while bone turnover is well-known to be reduced in patients with T2DM. We tested the hypothesis that postmenopausal women with T2DM have lower BTM and lower cortical porosity than those without diabetes, and that higher serum levels of glucose and BMI are associated with lower BTM and with lower cortical porosity (115).

This cross-sectional study was based on a prior nested case-control study including 443 postmenopausal women aged 54–94 years from the Tromsø Study, 211 with nonvertebral fracture and 232 fracture-free controls. Of these 443 participants, 22 women had T2DM and 421 women did not have diabetes. All had fasting blood samples assayed for PINP, CTX, and glucose, and femoral subtrochanteric architecture quantified from clinical CT images.

Women with T2DM had higher serum glucose, BMI, and femoral subtrochanteric total and cortical vBMD but lower cortical porosity than nondiabetic women. Increased glucose level was associated with lower PINP, CTX, and cortical porosity, while increased BMI was associated with lower serum PINP and CTX and thicker cortices.

Increasing glucose level and BMI were associated with lower bone turnover. Intracortical and endocortical remodeling lead to reduced porosity and thicker cortices. Cortical porosity was lower in women with T2DM than in women without diabetes. This indicated that other changes in bone qualities rather than increased cortical porosity are likely to explain the increased fracture risk in patients with T2DM.

**Table 7.** Characteristics of postmenopausal women by fracture status

| | Cases (n = 211) | Controls (n = 232) | p-value |
|---|---|---|---|
| Age (year) | 68.4 ± 7.7 | 68.3 ± 6.7 | 0.937 |
| Height (cm) | 162.7 ± 6.1 | 161.2 ± 6.6 | 0.011 |
| Weight (kg) | 68.9 ± 10.5 | 70.0 ± 10.8 | 0.280 |
| Body mass index (kg/m²) | 26.0 ± 3.8 | 27.0 ± 4.3 | 0.015 |
| Self-reported good health, n (%) | 147 (70.3) | 165 (71.1) | 0.860 |
| Physical activity (hour/week) | 2.6 ± 1.6 | 2.5 ± 1.7 | 0.421 |
| Currently smoker, n (%) | 29 (13.7) | 24 (10.3) | 0.257 |
| Alcohol intake (drink/week) | 3.2 ± 3.7 | 3.3 ± 3.5 | 0.407 |
| History of previous fracture, n (%) | 61 (28.9) | 0 | |
| Parental hip fracture, n (%) | 34 (16.1) | 37 (16.0) | 0.469 |
| Rheumatoid arthritis, n (%) | 11 (5.2) | 8 (3.5) | 0.407 |
| Oral glucocorticoid use, n (%) | 8 (3.8) | 2 (0.9) | 0.023 |
| Take calcium supplements, n (%) | 44 (20.9) | 28 (12.1) | 0.007 |
| Take vitamin D supplements, n (%) | 163 (77.3) | 166 (71.6) | 0.278 |
| Hyperthyroidism, n (%) | 8 (3.8) | 6 (2.6) | 0.468 |
| Hypothyroidism, n (%) | 40 (19.0) | 20 (8.6) | 0.002 |
| Ulcerative colitis/Crohn's disease, n (%) | 12 (5.7) | 5 (2.2) | 0.054 |
| Diabetes, n (%) | 9 (4.3) | 13 (5.6) | 0.513 |
| Early menopause < 45 years, n (%) | 34 (16.1) | 22 (9.5) | 0.036 |
| eGFR (ml/min/1.73 m²) | 77.4 ± 16.8 | 77.8 ± 14.9 | 0.584 |
| eGFR below 60 ml/min/1.73 m²), n (%) | 25 (11.9) | 22 (9.5) | 0.409 |
| FN aBMD (mg/cm²) | 794 ± 100 | 860 ± 110 | <0.001 |
| FRAX estimate with FN aBMD (%) | 15.2 ± 7.8 | 10.8 ± 4.9 | <0.001 |
| Garvan estimate with FN aBMD (%) | 22.6 ± 13.3 | 14.4 ± 6.5 | <0.001 |
| Number of fracture >50 years, n (%)[*] | | | |
| 1 | 44 (20.9) | 0 | |
| 2 | 15 (7.1) | 0 | |
| ≥ 3 | 2 (1.0) | 0 | |
| Number of falls in past year, n (%) | | | |
| 0 | 138 (65.4) | 147 (63.4) | |
| 1 | 58 (27.5) | 71 (30.6) | |
| 2 | 14 (6.6) | 12 (5.2) | |
| ≥ 3 | 1 (0.5) | 2 (0.9) | |
| Femoral subtrochanter architecture | | | |
| Total bone vBMD (mg HA/cm³) | 684 ± 113 | 750 ± 90.0 | <0.001 |
| Cortical porosity (%) | 43.8 ± 4.35 | 41.7 ± 3.39 | <0.001 |
| Cortical thickness (mm) | 4.06 ± 0.58 | 4.36 ± 0.54 | <0.001 |
| Cortical cross-sectional area (mm²) | 409 ± 39.1 | 417 ± 39.4 | 0.029 |
| Cortical vBMD (mg HA/cm³) | 1025 ± 72.6 | 1059 ± 56.6 | <0.001 |
| Cortical bone mineral content (mg HA) | 1552 ± 184 | 1636 ± 174 | <0.001 |
| Trabecular BV/TV (%) | 0.266 ± 0.241 | 0.272 ± 0.314 | 0.806 |

Numbers represent means ± SDs or numbers (%).[*]The total number of fractures did not include index fractures. Cases and controls were compared using analysis of variance adjusted for age. FN, femoral neck; eGFR, estimated glomerular filtration rate; aBMD, areal bone mineral density; vBMD, volumetric BMD; HA, hydroxyapatite; BV/TV, bone volume/tissue volume; FRAX, Fracture Risk Assessment Tool for calculation of 10-year probability of major fracture; Garvan, Fracture Risk estimate of 10-year fracture risk for any fragility fracture.

# 5 Discussion

## 5.1 Methodological considerations

### 5.1.1 Internal validity

The internal validity of a study refers to whether the results are valid for the source population (116). Many types of bias can threaten the internal validity of an epidemiological study, and they can be classified into three main categories: selection bias, information bias, and confounding (117).

**Selection bias**

Case-control studies are efficient in identifying associations between exposure and outcome, useful to generate hypotheses, easy to organize, and less time consuming and expensive than cohort studies. However, case-control studies are prone to selection bias (118), which is defined as "a systematic error in a study that stems from the procedures used to select subjects and from factors that influence study participation" (117). One challenge is to select controls who are representative of women without the event of interest. By using a nested case-control design, we reduced the likelihood of selection bias as both cases and controls were recruited from the same well-defined Tromsø 4 and they were likely to be representative for the general Tromsø population (104, 119). The fracture-free age-matched controls were randomly selected from the Tromsø 4 cohort. We struggled to find fracture-free controls over 85 years of age. Previous studies have reported that participants of the Tromsø study, similar to those of other population-based studies, tend to be healthier, have healthier lifestyles, and be more educated than non-participants (104, 119). As the Tromsø study is based on the general population and has a high response rate, the risk of selection bias is likely small. In the present study, a pre-screening phone call was used to determine the eligibility of patients who responded and were willing to participate based on our inclusion and exclusion criteria. Some of them signaled during phone calls that despite their willingness, they could not participate because of health problems. The most severely ill women with fractures may therefore be underrepresented. Because of the tendency toward "healthy" selection bias, the association between cortical bone parameters and fracture risk could be underestimated.

**Non-responder bias**

One major concern in epidemiologic studies is non-responder bias because it could compromise the validity of the study. The association between exposure and disease might differ between

those who participate and those who do not participate in a study. Non-participants tend to have poorer health, lower socioeconomic status, and higher mortality, as demonstrated in previous studies (120, 121). There are conflicting conclusions regarding the impact of non-response on the results with some studies showing modest effects (121, 122) and others claiming that non-response does not cause bias in the associations studied (123).

To address non-responder bias, we used information from the Tromsø 4 survey (1994–95) regarding all 760 fracture patients who were invited to participate in this study (Fig. 5). We compared the characteristics in the 264 women who attended with those of the 496 who did not attend (Table 8). Non-participants were older, shorter, had lower BMI, were less physically active, believed that they were less healthy, and had a lower education level. This agrees with the previous findings from other surveys in the Tromsø Study, which reported a tendency of "healthy" selection bias when responders were compared with non-responders (119).

**Table 8.** Characteristics of Tromsø 4 (1994–95) fracture cases who participated and did not participate among all 760 invited fracture cases

|  | Participants n = 264 | Non-participants n = 496 |  |
| --- | --- | --- | --- |
|  | Mean (SD) | Mean (SD) | *p*-value |
| Age (years) | 51.2 (8.0) | 59.3 (10.5) | <0.001 |
| Height (cm) | 164.4 (5.7) | 162.1 (6.3) | <0.001 |
| Weight (kg) | 65.6 (9.8) | 67.0 (11.4) | 0.115 |
| Body mass index (kg/m$^2$) | 24.3 (3.5) | 25.5 (4.2) | <0.001 |
| Physical activity score* | 3.6 (2.2) | 2.8 (2.2) | <0.001 |
| Smoking, % | 29.8 | 30.9 | 0.760 |
| Self-perceived excellent/good health, % | 70.2 | 57.3 | <0.001 |
| Previous hip fracture, % | 0.4 | 4.1 | <0.001 |
| Previous wrist fracture, % | 16.9 | 24.3 | 0.027 |
| Education > 7–10 years, % | 64.9 | 41.4 | <0.001 |

*Physical activity score, hours of moderate activity + 2 × hours of hard activity
SD, standard deviation.

**Information bias and misclassification**

Information bias in epidemiologic studies results from systematic error in the study because the information collected about or from study participants is erroneous (117). Information bias involves the misclassification of the exposure or outcome resulting in under or overestimation of exposure or disease prevalence leading to incorrect estimates of associations between exposure and outcome. This can arise because of recall or reporting bias, observer bias, or imprecise or poorly calibrated instruments.

Misclassification occurs when participants included in the study are erroneously placed with respect to their exposure or outcome categories (118). Misclassification can be nondifferential or differential. "For exposure misclassification, the misclassification is nondifferential if it is unrelated to the occurrence or presence of disease; if the misclassification of exposure is different for those with and without disease, it is differential" (117). A misclassification that is the same in cases as in controls is nondifferential. Nondifferential misclassification usually dilutes the association, and a potentially true association may therefore not be detected. In contrast, in differential misclassification, an association can be either under or overestimated (117).

Recall bias is one example of differential misclassification that may occur if participants with fracture remember differently than participants without fractures in case-control studies. Another example is if the porosity threshold $> 80^{th}$ percentile as well as the other selected thresholds have caused a nondifferential error; however, nondifferential measurement errors usually dilute the risk estimate.

**Outcomes - fracture status**

In the current study, the fracture status was unlikely to be misclassified because the information on fracture status was obtained from a validated X-ray based fracture registry (107). The X-ray-based registry had much better sensitivity for fractures than did self-reported questionnaires. There was no over-reporting but a minor under-reporting of fractures in the X-ray based archives, which probably represents modest nondifferential misclassification without effects on results. In addition, pre-screening phone calls were made to confirm fracture status, and for confirmation of the fracture-free status of the controls (Appendix A).

**Questionnaire data**

The quality of the data obtained by self-administered questionnaires is depending on the recall ability of the participants. Tromsø study participants has shown to rate their leisure activity level in accord with their objectively measured (124). All participants (cases and controls) filled in information about their fracture status at enrollment. There was some inconsistency in the information obtained regarding the self-reported numbers of fractures and previous or subsequent fractures as explained in the Methods section and shown in Table 2. Therefore, we validated the "previous or subsequent" fractures in 91 (43%) of 211 fracture cases through medical records to avoid the misclassification of women with FRAX and Garvan estimates

below or above the selected thresholds used in this study. After the update of information about previous/subsequent fracture, the recalculated FRAX and Garvan estimates changed only modestly, as reported in Paper I. In addition, the exact number of falls during the last year can be hard to remember accurately.

In Paper III, the diagnosis of T2DM, duration of disease, and medication and fractures were confirmed through medical records to avoid misclassification. Premenopausal women and those using bisphosphonates or hormone replacement therapy were excluded because these factors influence both bone architecture and BTM and we wanted to avoid misclassification.

**Bone Measurements**

Measurement of the total hip and FN aBMD using DXA has good precision, with a CV of 1.2-1.7%. The measurements of bone architecture at the subtrochanteric region in clinical CT images, analyzed using StrAx1.0 software, also had good precision, with a CV of 0.3-2.3% (96).

The accuracy of porosity measurements in clinical CT images with a voxel size of 740 µm was validated by testing the agreement with HR-pQCT measurements with a voxel size of 82 µm as the gold standard (96, 112). The agreement ($R^2$) between CT and HR-pQCT measurements ranged from 0.86 to 0.94 for the quantification of porosity at the same femoral subtrochanteric site (porosity range 40–95%) using StrAx1.0 software (112). This confirmed strong correlations between CT and HR-pQCT measurement techniques. Furthermore, the difference between CT and HR-pQCT measurements of porosity ranged from 0% to 10% depending on the bone compartment (112). The *in vivo* and *ex vivo* precision was < 4% (96, 111, 125).

One limitation of this approach is that the StrAx1.0 software used to assess cortical bone parameters is sensitive to motion artefacts. For this reason, 66 participants were excluded from this study, as reported in the Methods. Of these 66 women who were excluded owing to motion artefacts, 49 (74.2%) were fracture cases and 17 were controls (25.8%). These 49 excluded cases were 3.2 years older than the 215 cases without motion artefacts (71.6 vs. 68.3 years), whereas the 17 excluded controls were not significantly older than the 246 controls without motion artefacts (69.2 vs. 68.2 years). The exclusion of these relatively older fracture cases with motion artefacts, resulted in good age-matching between the cases and controls. In fact, this solved the concern mentioned above regarding the challenge of finding sufficient numbers of

fracture-free controls in the upper 5-year age groups. For this reason, the cases and controls in the final analyses were of the same average age and thus the age-matching was good.

**Confounding and interaction**

"Confounding (from the Latin meaning "to pour together") is the confusion of two supposedly causal variables, so that part or all of the purported effect of one variable is actually due to the other" (126). The confounding variable must be associated with both the exposure and the outcome variable to create bias. One way to avoid confounding is to match the case and control groups with respect to possible confounders such as age (118). As age is one of the most important risk factors for fragility fractures, we used randomly selected age-matched controls to minimize confounding by age. In statistical analysis, the "change-in-estimate criterion" was used to identify confounders by comparing the estimated measure of the associations before and after adjusting the model for the potential confounder (118). To avoid confounding bias, we included potential confounding variables as covariates in the multivariable linear and logistic regression analyses (Paper I-III). We adjusted the models for age, height, weight, FN aBMD, FRAX, or Garvan because these factors are well-known to be associated with both bone traits and fracture risk. However, there might be other possible confounding factors that could influence the associations that were not adjusted for.

Interaction or effect modification occurs when an association between two variables differs according to a third variable. We included interaction terms in the logistic regression models to evaluate whether the effect of cortical porosity on fracture risk was modified by FN aBMD, FRAX, or Garvan estimates in Paper I. There was no interaction between these variables (all $p$ > 0.10); however, the lack of a significant interaction term does not necessarily exclude interactions.

**5.1.2 External validity or generalizability**

External validity or generalizability refers to whether the results are valid for other populations. The study participants in our studies were postmenopausal women aged 54–94 years old and all were Caucasian. Tromsø is the largest city in Northern Norway, north of the Arctic Circle, at a latitude of 69°N. This latitude may play role in cutaneous vitamin D production as the sun is below the horizon for two months in the winter, which can influence serum vitamin D levels.

It has been reported previously that there are geographic and regional differences in BMD and fracture risk in Norway (127, 128). However, elderly women (≥ 60 years) residing in Tromsø are reported to have only marginally higher age-adjusted BMD compared with women living in Bergen (South Norway) (128). The regional differences in fracture rate are most apparent between rural and urban areas, with lower hip and forearm fracture rates in rural areas (127, 129-132). In general, the Tromsø population is like the general Norwegian population and we believe that the results of this study are valid for Caucasian postmenopausal women in Norway and other western countries with the same age, gender, and ethnicity.

Comparing the results of cortical porosity measurements between different populations might be challenging because of the different techniques used in assessing porosity. The cortical porosity values of the proximal femur presented in this study are similar to the values in the proximal femur reported in another multicenter study of 50 postmenopausal women (112) and a study of cortical porosity of the distal radius in an Australian cohort of 345 women aged 40–60 years using the same StrAx1.0 software (31). As we in Norway and Scandinavia has higher rate of fracture, further studies are needed in other populations.

## 5.2 Significance of results
### 5.2.1 FRAX, Garvan and cortical porosity
In Paper I, we reported that cortical porosity and thickness remained associated with fracture even after adjusting for FRAX or Garvan estimates and identified additional fracture cases than those unidentified by FRAX or Garvan alone, with an increase in the AUC. Moreover, cortical porosity improved the net reclassification of women with fracture compared with FRAX alone. When cortical porosity and thickness were combined in the same models with FRAX and Garvan, cortical thickness was no longer associated with fracture independent of cortical porosity. This indicates that cortical porosity may be the most important cortical parameter associated with fracture risk (114).

The benefit and novelty of using the non-threshold-based software used in this study is how this differs from the traditional morphological assessment of porosity. The porosity values presented here were determined using a density-based software that quantifies the void fraction and is not a visually quantifiable estimate based on size and dimension. The measure of porosity was more inclusive by encompassing porosity of both the compact cortex and the TZ and by taking into account the partial volume effect. As a result, the values of porosity were higher (95, 96, 111,

125, 133, 134) than those in reports using other methods (97-99). The studies using HR-pQCT to quantify porosity have presented values within the 1–15-% range and this is likely due to only quantifying the porosity of the compact cortex and the porosity of completely empty voxels (97-99); thus, this threshold-based image analysis underestimates porosity (111, 135).

To our knowledge, there are only two prospective studies that have evaluated the predictive role of cortical porosity on incident fractures (99, 100). Ohlsson *et al.* reported that cortical area and mass but not porosity at the distal tibia predicted any type of fracture in older men assessed using HR-pQCT (99). The lack of an association of cortical porosity with incident fracture was in contrast to the results of previous studies, which reported that cortical porosity was associated with prevalent fractures in cross-sectional studies (31, 95-98, 136). In a recently published study, cortical porosity was quantified using two different methods: i) the HR-pQCT threshold-based morphological assessment method, and ii) the non-thresholding density-based StrAx1.0 software, which is the same as the method used in the current study (100). The authors reported that cortical porosity of the inner TZ at the ultra-distal radius was associated with incident major osteoporotic fractures in postmenopausal women after adjustment for FN aBMD and FRAX score. However, this association was attenuated and marginal after adjustment for ultra-distal radius aBMD ($p = 0.054$) (100). This discrepancy in the association of cortical porosity with fractures between cross-sectional and prospective studies might be due to the relatively short follow-up time and low number of fracture cases in the prospective studies. Additional prospective studies are needed to determine if cortical porosity predicts fracture.

**5.2.2 Patient profile in fracture cases identified by high cortical porosity**
In Paper II, we reported that of the 75% of fracture cases that were unidentified by FRAX, 20% were identified by cortical porosity and had a different patient profile from those identified by FRAX alone. Those who were identified by cortical porosity alone were younger and had a higher FN aBMD, a relatively larger bone size, a larger medullary cavity, and thinner and more porous cortices at the femoral subtrochanteric site than those identified by FRAX alone; in addition fewer had a prior fracture and parental history of hip fracture. Thus, the measurement of cortical porosity may capture additional fracture risk components that are not captured by FRAX. This may be of clinical benefit to identify women before they have their first fracture and thus useful for primary fracture prevention.

Bone needs to be strong to resist breaking and yet light to allow movement (21). To achieve the highest possible strength using the minimum net amount of bone, bone is shaped by modifying its mass distribution instead of increasing its mass (68). Wider bones with a thinner cortex are more resistant to fracture because the thinner cortex (with the same cortical area) is distributed further outward from the neutral axis (66, 73). For this reason, one could expect that these fracture cases with larger bone size and higher CSMI have stronger bones and were more resistant to fracture (73, 137). In contrast, our findings indicate that the advantages of having larger bones did not offset the disadvantages of their increased porosity. High cortical porosity can be seen as giant pores in cross-sectional images and the presence of large coalesced pores. The presence of large coalesced pores increases the risk of crack propagation and fracture, especially under tensile loading (138). This is supported by results from Turnbull *et al.*, who indicated that a microcrack located close to intracortical pores can compromise fracture resistance (139).

Cases identified by porosity measurements had significantly higher porosity in both the compact-appearing cortex and the outer TZ compared to the other three groups in this study. One possible explanation for this is that increased porosity in the outer part of the cortex (more distant from the neutral axis) might cause a greater loss of bending strength than if the increased porosity is located closer to the neutral axis (140). As bending is imposed, the stress distribution in tubular bone is not uniform at any particular cross-section; it is zero at the neutral axis, becomes gradually greater, and is at its highest at the outer surfaces of a bone (141). Given the high stress on the outer part of the cortex during trauma, the increased porosity at this location might contribute to the increased fracture risk in the fracture cases identified by high cortical porosity. However, this needs to be studied further. Interestingly, the fracture cases who were identified by high cortical porosity were younger and tended to be healthier, albeit without statistical significance. One possible reason is that genetic variation in bone traits are established during growth early in life and may contribute to fracture risk in the early years of life.

### 5.2.3 Type 2 diabetes mellitus and cortical porosity

In Paper III, we reported that 22 women with T2DM had higher serum glucose, BMI, and higher femoral subtrochanteric total vBMD but lower cortical porosity than 421 nondiabetic women. Increasing serum glucose was associated with lower BTM and lower cortical porosity. We

inferred from these results that other changes in bone qualities rather than increased cortical porosity are likely to explain the increased fracture propensity in patients with T2DM (115).

Patients with T2DM have a modestly increased risk of any fragility fracture despite normal or increased aBMD, higher BMI, and low bone turnover, and would therefore be expected to have a reduced risk for fracture (142-146). The reasons for the increased risk of fracture in patients with T2DM is not well understood and is likely to be multifactorial. Increased cortical porosity at the distal radius and distal tibia, assessed using HR-pQCT, has been invoked as one possible factor (101-103). However, it is hard to explain how individuals with T2DM can have high cortical porosity. In contrast to previous results (101-103), we report lower cortical porosity at the femoral shaft in women with T2DM, assessed using low-resolution CT and Strax1.0 software.

Patients with fracture have increased bone turnover reflected by increased levels of BTM (125, 147). Increased intracortical remodeling along the Haversian canals produces increased cortical porosity, as shown in biopsies from the hip (69), in HR-pQCT images of the distal radius and distal tibia (31, 95), and in low-resolution images of the femoral shaft in fracture patients (96).

In this study, both increasing BMI and serum glucose level were associated with reduced levels of BTM, suggesting that reduced intracortical and endocortical remodeling may lead to reduced cortical porosity and thicker cortices. T2DM is associated with low bone turnover (145). Cortical porosity at the femoral subtrochanteric region was lower in the 22 women with T2DM compared to the 421 women without diabetes. In agreement with our findings, another larger study reported lower cortical porosity at the distal radius but not at the distal tibia using HR-pQCT in 99 women with T2DM compared to 954 controls aged 75–80 years (148).

We inferred that increasing cortical porosity is unlikely to explain the increased fracture risk in women with T2DM, and other alterations in other bone qualities rather than increased porosity are more likely to explain the increased fracture propensity in patients with T2DM.

# 6 Conclusions, implications, future perspectives

## 6.1 Conclusions

In Paper I, we examined the association of each of the single components included in the FRAX and Garvan tools (as chronic diseases) and femoral subtrochanteric parameters with odds for fracture. In Paper II, we explored further the results from Paper I by identifying the characteristics of those additional women with fracture who were unidentified by FRAX but identified by the measurement of cortical porosity. In Paper III, we performed a sub-group analysis of those women who had one of the most common chronic disease, T2DM, and those who had a fracture in order to increase our understanding of the pathophysiology behind their fragility fractures.

We examined whether the inclusion of cortical parameters such as cortical porosity, thickness, and area could provide additional information about fracture risk beyond that provided by existing tools such as FRAX and Garvan. These results are novel in reporting that cortical porosity was associated with increased fracture risk after three methods of calculation: i) odds ratio, ii) AUC, and iii) NRI. After exploring other cortical bone parameters, cortical porosity was the most important cortical parameter associated with fracture in these data. The results indicated that cortical porosity may captures additional risk, and may be a potential and suitable predictor of fracture risk.

Women with fracture who were unidentified by FRAX but identified by high cortical porosity had a different patient profile than those identified by FRAX alone. This finding is novel. These women were younger, had higher FN aBMD and lower FRAX score, and had an architecture in which the positive impact of larger bone size did not offset the negative effect of thinner cortices with increased porosity.

We reported that higher BMI and serum glucose were associated with lower BTM and cortical porosity. These results suggest that increasing cortical porosity is unlikely to explain the increased fracture risk in women with T2DM, and that other alterations in other bone qualities rather than increased porosity are more likely to explain the increased fracture propensity in patients with T2DM. This as among the first report to reveal lower cortical porosity in women with T2DM. This finding is therefore relatively novel.

**6.2 Implications and further research**

As cortical porosity improved the net reclassification of women with fractures, this measurement is likely to predict fracture and help improve the identification of women who are at risk of fracture. The assessment of cortical porosity may be particularly useful for the identification of fracture risk in individuals without osteoporosis and in those with a low FRAX score. Adding cortical porosity to existing tools may improve their predictive performance.

Improving sensitivity for fracture clearly remains a challenge as half of cases were still not identified. It is likely that future tools will need to include bone architectural parameters and non-skeletal and genetic properties in addition to clinical risk factors to achieve better fracture risk prediction.

Most importantly, further and larger prospective studies are needed to determine whether cortical parameters truly predict fractures. If this hypothesis is valid, studies will be needed to determine treatment thresholds for cortical porosity. The development of clinical procedures for scanning and analyzing images with a low demand on facilities will be required.

As there are only about 70 HR-pQCT machines worldwide, this technology is not widely accessible in clinical practice. Further research that takes advantage of using the widely available CT scanner may be beneficial and will preferably involve scanning of a central site.

# References

1.  Johnell O, Kanis JA. An estimate of the worldwide prevalence and disability associated with osteoporotic fractures. Osteoporos Int. 2006;17(12):1726-33.

2.  Hernlund E, Svedbom A, Ivergard M, Compston J, Cooper C, Stenmark J, et al. Osteoporosis in the European Union: medical management, epidemiology and economic burden. A report prepared in collaboration with the International Osteoporosis Foundation (IOF) and the European Federation of Pharmaceutical Industry Associations (EFPIA). Arch Osteoporos. 2013;8:136.

3.  Cummings SR, Melton LJ. Epidemiology and outcomes of osteoporotic fractures. Lancet. 2002;359(9319):1761-7.

4.  Sambrook P, Cooper C. Osteoporosis. Lancet. 2006;367(9527):2010-8.

5.  Svedbom A, Hernlund E, Ivergard M, Compston J, Cooper C, Stenmark J, et al. Osteoporosis in the European Union: a compendium of country-specific reports. Arch Osteoporos. 2013;8:137.

6.  Dhanwal DK, Dennison EM, Harvey NC, Cooper C. Epidemiology of hip fracture: Worldwide geographic variation. Indian J Orthop. 2011;45(1):15-22.

7.  Kanis JA, Oden A, McCloskey EV, Johansson H, Wahl DA, Cooper C. A systematic review of hip fracture incidence and probability of fracture worldwide. Osteoporos Int. 2012;23(9):2239-56.

8.  Lofthus CM, Frihagen F, Meyer HE, Nordsletten L, Melhuus K, Falch JA. Epidemiology of distal forearm fractures in Oslo, Norway. Osteoporos Int. 2008;19(6):781-6.

9.  Omsland TK, Holvik K, Meyer HE, Center JR, Emaus N, Tell GS, et al. Hip fractures in Norway 1999-2008: time trends in total incidence and second hip fracture rates: a NOREPOS study. Eur J Epidemiol. 2012;27(10):807-14.

10. Falch JA, Meyer HE. Osteoporosis and fractures in Norway. Occurrence and risk factors. Tidsskr Nor Legeforen. 1998;118(4):568-72.

11. Statistics Norway 2016 [Available from: www.ssb.no].

12. Søgaard AJ, Holvik K, Meyer HE, Tell GS, Gjesdal CG, Emaus N, et al. Continued decline in hip fracture incidence in Norway: a NOREPOS study. Osteoporos Int. 2016;27(7):2217-22.

13. Hoff M, Torvik IA, Schei B. Forearm fractures in Central Norway, 1999-2012: incidence, time trends, and seasonal variation. Arch Osteoporos. 2016;11:7.

14. Johnell O, Kanis J. Epidemiology of osteoporotic fractures. Osteoporos Int. 2005;16 Suppl 2:S3-7.

15. Kanis JA, Johnell O, Oden A, Sembo I, Redlund-Johnell I, Dawson A, et al. Long-term risk of osteoporotic fracture in Malmo. Osteoporos Int. 2000;11(8):669-74.

16. Borgström F, Sobocki P, Ström O, Jonsson B. The societal burden of osteoporosis in Sweden. Bone. 2007;40(6):1602-9.

17. Hansen L, Mathiesen AS, Vestergaard P, Ehlers LH, Petersen KD. A health economic analysis of osteoporotic fractures: who carries the burden? Arch Osteoporos. 2013;8:126.

18. Hektoen LF. Kostnader ved hoftebrudd hos eldre. HiOA Rapport 2014 nr3. Oslo:Høgskolen i Oslo og Akershus, 2014, p 41. [Available from:www.helsedirektoratet.no].

19. Melton LJ, 3rd, Chrischilles EA, Cooper C, Lane AW, Riggs BL. How many women have osteoporosis? JBMR Anniversary Classic. JBMR, Volume 7, Number 9, 1992. J Bone Miner Res. 2005;20(5):886-92.

20. Holroyd C, Cooper C, Dennison E. Epidemiology of osteoporosis. Best Pract Res Clin Endocrinol Metab. 2008;22(5):671-85.

21. Seeman E. Pathogenesis of bone fragility in women and men. Lancet. 2002;359(9320):1841-50.

22. Marshall D, Johnell O, Wedel H. Meta-analysis of how well measures of bone mineral density predict occurrence of osteoporotic fractures. BMJ. 1996;312(7041):1254-9.

23. Schuit SC, van der Klift M, Weel AE, de Laet CE, Burger H, Seeman E, et al. Fracture incidence and association with bone mineral density in elderly men and women: the Rotterdam Study. Bone. 2004;34(1):195-202.

24. Johnell O, Kanis JA, Oden A, Johansson H, De Laet C, Delmas P, et al. Predictive value of BMD for hip and other fractures. J Bone Miner Res. 2005;20(7):1185-94.

25. De Laet C, Kanis JA, Oden A, Johanson H, Johnell O, Delmas P, et al. Body mass index as a predictor of fracture risk: a meta-analysis. Osteoporos Int. 2005;16(11):1330-8.

26. Kanis JA. Diagnosis of osteoporosis and assessment of fracture risk. Lancet. 2002;359(9321):1929-36.

27. Kanis JA, Johnell O, De Laet C, Johansson H, Oden A, Delmas P, et al. A meta-analysis of previous fracture and subsequent fracture risk. Bone. 2004;35(2):375-82.

28. Kanis JA, Johansson H, Oden A, Johnell O, De Laet C, Eisman JA, et al. A family history of fracture and fracture risk: a meta-analysis. Bone. 2004;35(5):1029-37.

29. Gunnes M, Lehmann EH, Mellström D, Johnell O. The relationship between anthropometric measurements and fractures in women. Bone. 1996;19(4):407-13.

30. Joakimsen RM, Fønnebø V, Magnus JH, Tollan A, Søgaard AJ. The Tromsø Study: body height, body mass index and fractures. Osteoporos Int. 1998;8(5):436-42.

31. Bjørnerem Å, Bui QM, Ghasem-Zadeh A, Hopper JL, Zebaze R, Seeman E. Fracture risk and height: an association partly accounted for by cortical porosity of relatively thinner cortices. J Bone Miner Res. 2013;28(9):2017-26.

32. Ralston SH. Genetics of osteoporosis. Ann N Y Acad Sci. 2010;1192:181-9.

33. Bjørnerem Å, Bui M, Wang X, Ghasem-Zadeh A, Hopper JL, Zebaze R, et al. Genetic and environmental variances of bone microarchitecture and bone remodeling markers: a twin study. J Bone Miner Res. 2015;30(3):519-27.

34. Schwartz AV, Nevitt MC, Brown BW, Jr., Kelsey JL. Increased falling as a risk factor for fracture among older women: the study of osteoporotic fractures. Am J Epidemiol. 2005;161(2):180-5.

35. Cummings SR, Nevitt MC, Browner WS, Stone K, Fox KM, Ensrud KE, et al. Risk factors for hip fracture in white women. Study of Osteoporotic Fractures Research Group. N Engl J Med. 1995;332(12):767-73.

36. Boonen S, Dejaeger E, Vanderschueren D, Venken K, Bogaerts A, Verschueren S, et al. Osteoporosis and osteoporotic fracture occurrence and prevention in the elderly: a geriatric perspective. Best Pract Res Clin Endocrinol Metab. 2008;22(5):765-85.

37. Kim SY, Schneeweiss S, Liu J, Daniel GW, Chang CL, Garneau K, et al. Risk of osteoporotic fracture in a large population-based cohort of patients with rheumatoid arthritis. Arthritis Res Ther. 2010;12(4):R154.

38. Starup-Linde J, Frost M, Vestergaard P, Abrahamsen B. Epidemiology of Fractures in Diabetes. Calcif Tissue Int. 2017;100(2):109-21.

39. Vestergaard P, Rejnmark L, Mosekilde L. Influence of hyper- and hypothyroidism, and the effects of treatment with antithyroid drugs and levothyroxine on fracture risk. Calcif Tissue Int. 2005;77(3):139-44.

40. Kanis JA. Assessment of fracture risk and its application to screening for postmenopausal osteoporosis: synopsis of a WHO report. WHO Study Group. Osteoporos Int. 1994;4(6):368-81.

41. Siris ES, Chen YT, Abbott TA, Barrett-Connor E, Miller PD, Wehren LE, et al. Bone mineral density thresholds for pharmacological intervention to prevent fractures. Arch Intern Med. 2004;164(10):1108-12.

42. Pasco JA, Seeman E, Henry MJ, Merriman EN, Nicholson GC, Kotowicz MA. The population burden of fractures originates in women with osteopenia, not osteoporosis. Osteoporos Int. 2006;17(9):1404-9.

43. Consensus development conference: diagnosis, prophylaxis, and treatment of osteoporosis. Am J Med. 1993;94(6):646-50.

44. Organization WHO. Guidelines for preclinical evaluation and clinical trials in osteoporosis. Geneva; 1998.

45. Kanis JA, Borgström F, De Laet C, Johansson H, Johnell O, Jonsson B, et al. Assessment of fracture risk. Osteoporos Int. 2005;16(6):581-9.

46. Aspray TJ. Fragility fracture: recent developments in risk assessment. Ther Adv Musculoskelet Dis. 2015;7(1):17-25.

47. Kanis JA, Johnell O, Oden A, Johansson H, McCloskey E. FRAX and the assessment of fracture probability in men and women from the UK. Osteoporos Int. 2008;19(4):385-97.

48. Leslie WD, Brennan SL, Lix LM, Johansson H, Oden A, McCloskey E, et al. Direct comparison of eight national FRAX(R) tools for fracture prediction and treatment qualification in Canadian women. Arch Osteoporos. 2013;8:145.

49. McCloskey EV, Oden A, Harvey NC, Leslie WD, Hans D, Johansson H, et al. Adjusting fracture probability by trabecular bone score. Calcif Tissue Int. 2015;96(6):500-9.

50. McCloskey EV, Harvey NC, Johansson H, Kanis JA. FRAX updates 2016. Curr Opin Rheumatol. 2016;28(4):433-41.

51. Kanis JA, Harvey NC, Johansson H, Oden A, Leslie WD, McCloskey EV. FRAX and fracture prediction without bone mineral density. Climacteric. 2015;18 Suppl 2:2-9.

52. Kanis JA, Oden A, Johansson H, McCloskey E. Pitfalls in the external validation of FRAX. Osteoporos Int. 2012;23(2):423-31.

53. Kanis JA, Oden A, Johnell O, Johansson H, De Laet C, Brown J, et al. The use of clinical risk factors enhances the performance of BMD in the prediction of hip and osteoporotic fractures in men and women. Osteoporos Int. 2007;18(8):1033-46.

54. Nguyen ND, Frost SA, Center JR, Eisman JA, Nguyen TV. Development of prognostic nomograms for individualizing 5-year and 10-year fracture risks. Osteoporos Int. 2008;19(10):1431-44.

55. van Geel TA, Nguyen ND, Geusens PP, Center JR, Nguyen TV, Dinant GJ, et al. Development of a simple prognostic nomogram for individualising 5-year and 10-year absolute risks of fracture: a population-based prospective study among postmenopausal women. Ann Rheum Dis. 2011;70(1):92-7.

56. Ahmed LA, Nguyen ND, Bjørnerem Å, Joakimsen RM, Jørgensen L, Størmer J, et al. External validation of the Garvan nomograms for predicting absolute fracture risk: the Tromsø Study. PLoS One. 2014;9(9):e107695.

57. Langsetmo L, Nguyen TV, Nguyen ND, Kovacs CS, Prior JC, Center JR, et al. Independent external validation of nomograms for predicting risk of low-trauma fracture and hip fracture. CMAJ. 2011;183(2):E107-14.

58. Bolland MJ, Siu AT, Mason BH, Horne AM, Ames RW, Grey AB, et al. Evaluation of the FRAX and Garvan fracture risk calculators in older women. J Bone Miner Res. 2011;26(2):420-7.

59. van Geel TA, Eisman JA, Geusens PP, van den Bergh JP, Center JR, Dinant GJ. The utility of absolute risk prediction using FRAX(R) and Garvan Fracture Risk Calculator in daily practice. Maturitas. 2014;77(2):174-9.

60. Tortora GJ, Derrickson B. Principles of anatomy and physiology : 1 : Organization, support and movement, and control systems of the human body. 12th ed. New York: Wiley; 2009.

61. Burr DB, Akkus O. Basic and Applied Bone Biology. San Diego: Academic Press; 2014.

62. Clarke B. Normal bone anatomy and physiology. Clin J Am Soc Nephrol. 2008;3 Suppl 3:S131-9.

63. Seeman E, Delmas PD. Bone quality - the material and structural basis of bone strength and fragility. N Engl J Med. 2006;354(21):2250-61.

64. Datta HK, Ng WF, Walker JA, Tuck SP, Varanasi SS. The cell biology of bone metabolism. J Clin Pathol. 2008;61(5):577-87.

65. Crockett JC, Rogers MJ, Coxon FP, Hocking LJ, Helfrich MH. Bone remodelling at a glance. J Cell Sci. 2011;124(Pt 7):991-8.

66. Morgan EF, Barnes GL, Einhorn TA. The Bone Organ System: Form and Function In: Feldman D, Dempster DW, Luckey M, Cauley JA, editors. Osteoporosis. 4th ed. San Diego: Academic Press; 2013. p. 3-20.

67. Rosen CJe. Front Matter, in Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism. 8th ed: John Wiley & Sons, Inc., Ames, USA.; 2013.

68. Seeman E. Structural basis of growth-related gain and age-related loss of bone strength. Rheumatology (Oxford). 2008;47 Suppl 4:iv2-8.

69. Zebaze RM, Ghasem-Zadeh A, Bohte A, Iuliano-Burns S, Mirams M, Price RI, et al. Intracortical remodelling and porosity in the distal radius and post-mortem femurs of women: a cross-sectional study. Lancet. 2010;375(9727):1729-36.

70. Parfitt AM. Misconceptions (2): turnover is always higher in cancellous than in cortical bone. Bone. 2002;30(6):807-9.

71. Seeman E. Age- and menopause-related bone loss compromise cortical and trabecular microstructure. J Gerontol A Biol Sci Med Sci. 2013;68(10):1218-25.

72. Franz-Odendaal TA, Hall BK, Witten PE. Buried alive: how osteoblasts become osteocytes. Dev Dyn. 2006;235(1):176-90.

73. Bouxsein ML. Determinants of skeletal fragility. Best Pract Res Clin Rheumatol. 2005;19(6):897-911.

74. Seeman E. Principles of Bone Biology, Modeling and Remodeling. The Cellular Machinery Responsible for the Gain and Loss of Bone's Material and Structural Strength. 3rd ed: Academic Press; 2008.

75. Parfitt AM. Targeted and nontargeted bone remodeling: relationship to basic multicellular unit origination and progression. Bone. 2002;30(1):5-7.

76. Manolagas SC, Parfitt AM. What old means to bone. Trends Endocrinol Metab. 2010;21(6):369-74.

77. Rizzoli R, . Atlas of Postmenopausal Osteoporosis. 3rd ed: Springer Healthcare Ltd 2010.

78. Andersen TL, Sondergaard TE, Skorzynska KE, Dagnaes-Hansen F, Plesner TL, Hauge EM, et al. A physical mechanism for coupling bone resorption and formation in adult human bone. Am J Pathol. 2009;174(1):239-47.

79. Parfitt AM. The bone remodeling compartment: a circulatory function for bone lining cells. J Bone Miner Res. 2001;16(9):1583-5.

80. Hauge EM, Qvesel D, Eriksen EF, Mosekilde L, Melsen F. Cancellous bone remodeling occurs in specialized compartments lined by cells expressing osteoblastic markers. J Bone Miner Res. 2001;16(9):1575-82.

81. Szulc P, Seeman E. Thinking inside and outside the envelopes of bone: dedicated to PDD. Osteoporos Int. 2009;20(8):1281-8.

82. Orwoll ES. Toward an expanded understanding of the role of the periosteum in skeletal health. J Bone Miner Res. 2003;18(6):949-54.

83. Eastell R, Szulc P. Use of bone turnover markers in postmenopausal osteoporosis. Lancet Diabetes Endocrinol . 2017;(11):908-923.

84. Naylor K, Eastell R. Bone turnover markers: use in osteoporosis. Nat Rev Rheumatol. 2012;8(7):379-89.

85.  Hlaing TT, Compston JE. Biochemical markers of bone turnover - uses and limitations. Ann Clin Biochem. 2014;51(Pt 2):189-202.

86.  Vasikaran S, Eastell R, Bruyere O, Foldes AJ, Garnero P, Griesmacher A, et al. Markers of bone turnover for the prediction of fracture risk and monitoring of osteoporosis treatment: a need for international reference standards. Osteoporos Int. 2011;22(2):391-420.

87.  Johansson H, Odén A, Kanis JA, McCloskey EV, Morris HA, Cooper C, et al. A meta-analysis of reference markers of bone turnover for prediction of fracture. Calcif Tissue Int. 2014;94(5):560-7.

88.  van der Linden JC, Homminga J, Verhaar JA, Weinans H. Mechanical consequences of bone loss in cancellous bone. J Bone Miner Res. 2001;16(3):457-65.

89.  Qiu S, Rao DS, Fyhrie DP, Palnitkar S, Parfitt AM. The morphological association between microcracks and osteocyte lacunae in human cortical bone. Bone. 2005;37(1):10-5.

90.  Bell KL, Loveridge N, Power J, Garrahan N, Meggitt BF, Reeve J. Regional differences in cortical porosity in the fractured femoral neck. Bone. 1999;24(1):57-64.

91.  Bell KL, Loveridge N, Power J, Garrahan N, Stanton M, Lunt M, et al. Structure of the femoral neck in hip fracture: cortical bone loss in the inferoanterior to superoposterior axis. J Bone Miner Res. 1999;14(1):111-9.

92.  Turner CH. Biomechanics of bone: determinants of skeletal fragility and bone quality. Osteoporos Int. 2002;13(2):97-104.

93.  Turner CH. Bone strength: current concepts. Ann N Y Acad Sci. 2006;1068:429-46.

94.  Bala Y, Bui QM, Wang XF, Iuliano S, Wang Q, Ghasem-Zadeh A, et al. Trabecular and cortical microstructure and fragility of the distal radius in women. J Bone Miner Res. 2015;30(4):621-9.

95.  Bala Y, Zebaze R, Ghasem-Zadeh A, Atkinson EJ, Iuliano S, Peterson JM, et al. Cortical porosity identifies women with osteopenia at increased risk for forearm fractures. J Bone Miner Res. 2014;29(6):1356-62.

96.  Ahmed LA, Shigdel R, Joakimsen RM, Eldevik OP, Eriksen EF, Ghasem-Zadeh A, et al. Measurement of cortical porosity of the proximal femur improves identification of women with nonvertebral fragility fractures. Osteoporos Int. 2015;26(8):2137-46.

97.  Sundh D, Mellström D, Nilsson M, Karlsson M, Ohlsson C, Lorentzon M. Increased Cortical Porosity in Older Men With Fracture. J Bone Miner Res. 2015;30(9):1692-700.

98.  Sundh D, Nilsson AG, Nilsson M, Johansson L, Mellström D, Lorentzon M. Increased cortical porosity in women with hip fracture. J Intern Med. 2017;281(5):496-506.

99.  Ohlsson C, Sundh D, Wallerek A, Nilsson M, Karlsson M, Johansson H, et al. Cortical Bone Area Predicts Incident Fractures Independently of Areal Bone Mineral Density in Older Men. J Clin Endocrinol Metab. 2017;102(2):516-24.

100. Biver E, Durosier-Izart C, Chevalley T, van Rietbergen B, Rizzoli R, Ferrari S. Evaluation of radius microstructure and areal bone mineral density improves fracture prediction in postmenopausal women. J Bone Miner Res. 2017. 10.1002/jbmr.3299 [Epub ahead of print].

101. Burghardt AJ, Issever AS, Schwartz AV, Davis KA, Masharani U, Majumdar S, et al. High-resolution peripheral quantitative computed tomographic imaging of cortical and trabecular bone microarchitecture in patients with type 2 diabetes mellitus. J Clin Endocrinol Metab. 2010;95(11):5045-55.

102. Patsch JM, Burghardt AJ, Yap SP, Baum T, Schwartz AV, Joseph GB, et al. Increased cortical porosity in type 2 diabetic postmenopausal women with fragility fractures. J Bone Miner Res. 2013;28(2):313-24.

103. Paccou J, Ward KA, Jameson KA, Dennison EM, Cooper C, Edwards MH. Bone Microarchitecture in Men and Women with Diabetes: The Importance of Cortical Porosity. Calcif Tissue Int. 2016;98(5):465-73.

104. Jacobsen BK, Eggen AE, Mathiesen EB, Wilsgaard T, Njølstad I. Cohort profile: the Tromsø Study. Int J Epidemiol. 2012;41(4):961-7.

105. Ahmed LA, Center JR, Bjørnerem Å, Bluic D, Joakimsen RM, Jørgensen L, et al. Progressively increasing fracture risk with advancing age after initial incident fragility fracture: the Tromsø Study. J Bone Miner Res. 2013;28(10):2214-21.

106. Bjørnerem Å, Ahmed LA, Jørgensen L, Størmer J, Joakimsen RM. Breastfeeding protects against hip fracture in postmenopausal women: the Tromsø Study. J Bone Miner Res. 2011;26(12):2843-50.

107. Joakimsen RM, Fønnebø V, Søgaard AJ, Tollan A, Størmer J, Magnus JH. The Tromsø Study: registration of fractures, how good are self-reports, a computerized radiographic register and a discharge register? Osteoporos Int. 2001;12(12):1001-5.

108. Barrett JF, Keat N. Artifacts in CT: recognition and avoidance. Radiographics. 2004;24(6):1679-91.

109. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985;28(7):412-9.

110. Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF, 3rd, Feldman HI, et al. A new equation to estimate glomerular filtration rate. Ann Intern Med. 2009;150(9):604-12.

111. Zebaze R, Ghasem-Zadeh A, Mbala A, Seeman E. A new method of segmentation of compact-appearing, transitional and trabecular compartments and quantification of cortical porosity from high resolution peripheral quantitative computed tomographic images. Bone. 2013;54(1):8-20.

112. Zebaze R, Libanati C, McClung MR, Zanchetta JR, Kendler DL, Høiseth A, et al. Denosumab Reduces Cortical Porosity of the Proximal Femoral Shaft in Postmenopausal Women With Osteoporosis. J Bone Miner Res. 2016;31(10):1827-34.

113. Leening MJ, Vedder MM, Witteman JC, Pencina MJ, Steyerberg EW. Net reclassification improvement: computation, interpretation, and controversies: a literature review and clinician's guide. Ann Intern Med. 2014;160(2):122-31.

114. Kral R, Osima M, Borgen TT, Vestgaard R, Richardsen E, Bjørnerem Å. Increased cortical porosity and reduced cortical thickness of the proximal femur are associated with nonvertebral fracture independent of Fracture Risk Assessment Tool and Garvan estimates in postmenopausal women. PLoS One. 2017;12(9):e0185363.

115. Osima M, Kral R, Borgen TT, Høgestøl IK, Joakimsen RM, Eriksen EF, et al. Women with type 2 diabetes mellitus have lower cortical porosity of the proximal femoral shaft using low-resolution CT than nondiabetic women, and increasing glucose is associated with reduced cortical porosity. Bone. 2017;97:252-60.

116. Fletcher R, & Fletcher, SW. Clinical epidemiology : The essentials. 4th ed. Baltimore: Lippincott Williams & Wilkins; 2005.

117. Rothman K. Epidemiology : An introduction. 2nd ed. Oxford: Oxford University Press; 2012.

118. Szklo M N, FJ. Epidemiology: Beyond the basics. 3rd ed. Burlington: Jones & Barlett Learning; 2014.

119. Eggen AE, Mathiesen EB, Wilsgaard T, Jacobsen BK, Njølstad I. The sixth survey of the Tromsø Study (Tromsø 6) in 2007-08: collaborative research in the interface between clinical medicine and epidemiology: study objectives, design, data collection procedures, and attendance in a multipurpose population-based health survey. Scand J Public Health. 2013;41(1):65-80.

120. Knudsen AK, Hotopf M, Skogen JC, Øverland S, Mykletun A. The health status of nonparticipants in a population-based health study: the Hordaland Health Study. Am J Epidemiol. 2010;172(11):1306-14.

121. Langhammer A, Krokstad S, Romundstad P, Heggland J, Holmen J. The HUNT study: participation is associated with survival and depends on socioeconomic status, diseases and symptoms. BMC Med Res Methodol. 2012;12:143.

122. Søgaard AJ, Selmer R, Bjertness E, Thelle D. The Oslo Health Study: The impact of self-selection in a large, population-based survey. Int Equity Health. 2004;3(1):3.

123. Harald K, Salomaa V, Jousilahti P, Koskinen S, Vartiainen E. Non-participation and mortality in different socioeconomic groups: the FINRISK population surveys in 1972-92. J Epidemiol Community Health. 2007;61(5):449-54.

124. Emaus A, Degerstrøm J, Wilsgaard T, Hansen BH, Dieli-Conwright CM, Furberg AS, et al. Does a variation in self-reported physical activity reflect variation in objectively measured physical activity, resting heart rate, and physical fitness? Results from the Tromsø Study. Scand J Public Health. 2010;38(5 Suppl):105-18.

125. Shigdel R, Osima M, Ahmed LA, Joakimsen RM, Eriksen EF, Zebaze R, et al. Bone turnover markers are associated with higher cortical porosity, thinner cortices, and larger size of the proximal femur and non-vertebral fractures. Bone. 2015;81:1-6.

126. Katz D, Wild, D., Elmore, J., & Lucan, S. Jekel's Epidemiology, Biostatistics and Preventive Medicine Jekel's Epidemiology, Biostatistics and Preventive Medicine. 4th ed. London: Elsevier Health Sciences; 2013.

127. Falch JA, Kaastad TS, Bøhler G, Espeland J, Sundsvold OJ. Secular increase and geographical differences in hip fracture incidence in Norway. Bone. 1993;14(4):643-5.

128. Omsland TK, Gjesdal CG, Emaus N, Tell GS, Meyer HE. Regional differences in hip bone mineral density levels in Norway: the NOREPOS study. Osteoporos Int. 2009;20(4):631-8.

129. Finsen V, Benum P. Changing incidence of hip fractures in rural and urban areas of central Norway. Clin Orthop Relat Res. 1987(218):104-10.

130. Sanders KM, Nicholson GC, Ugoni AM, Seeman E, Pasco JA, Kotowicz MA. Fracture rates lower in rural than urban communities: the Geelong Osteoporosis Study. J Epidemiol Community Health. 2002;56(6):466-70.

131. Rosengren BE, Ahlborg HG, Gärdsell P, Sernbo I, Daly RM, Nilsson JA, et al. Bone mineral density and incidence of hip fracture in Swedish urban and rural women 1987-2002. Acta Orthop. 2010;81(4):453-9.

132. Søgaard AJ, Gustad TK, Bjertness E, Tell GS, Schei B, Emaus N, et al. Urban-rural differences in distal forearm fractures: Cohort Norway. Osteoporos Int. 2007;18(8):1063-72.

133. Shigdel R, Osima M, Lukic M, Ahmed LA, Joakimsen RM, Eriksen EF, et al. Determinants of Transitional Zone Area and Porosity of the Proximal Femur Quantified In Vivo in Postmenopausal Women. J Bone Miner Res. 2016;31(4):758-66.

134. Bjørnerem Å. The clinical contribution of cortical porosity to fragility fractures. BoneKey Rep. 2016;5:846.

135. Zebaze R, Seeman E. Cortical bone: a challenging geography. J Bone Miner Res. 2015;30(1):24-9.

136. Dhainaut A, Hoff M, Syversen U, Haugeberg G. Cortical hand bone porosity and its association with distal radius fracture in middle aged and elderly women. PLoS One. 2013;8(7):e68405.

137. Zebaze RM, Jones A, Knackstedt M, Maalouf G, Seeman E. Construction of the femoral neck during growth determines its strength in old age. J Bone Miner Res. 2007;22(7):1055-61.

138. Cooper DM, Kawalilak CE, Harrison K, Johnston BD, Johnston JD. Cortical Bone Porosity: What Is It, Why Is It Important, and How Can We Detect It? Curr Osteoporos Rep. 2016;14(5):187-98.

139. Turnbull TL, Baumann AP, Roeder RK. Fatigue microcracks that initiate fracture are located near elevated intracortical porosity but not elevated mineralization. J Biomech. 2014;47(12):3135-42.

140. Seeman E. Growth and Age-Related Abnormalities in Cortical Structure and Fracture Risk. Endocrinol Metab. (Seoul, Korea). 2015;30(4):419-28.

141. Currey J. Bones: Structure and mechanics: Princeton University Press; 2002.

142. Shanbhogue VV, Mitchell DM, Rosen CJ, Bouxsein ML. Type 2 diabetes and the skeleton: new insights into sweet bones. Lancet Diabetes Endocrinol. 2016;4(2):159-73.

143. Vestergaard P. Discrepancies in bone mineral density and fracture risk in patients with type 1 and type 2 diabetes - a meta-analysis. Osteoporos Int. 2007;18(4):427-44.

144. Rubin MR, Patsch JM. Assessment of bone turnover and bone quality in type 2 diabetic bone disease: current concepts and future directions. Bone Res. 2016;4:16001.

145. Starup-Linde J, Vestergaard P. Biochemical bone turnover markers in diabetes mellitus - A systematic review. Bone. 2016;82:69-78.

146. Napoli N, Chandran M, Pierroz DD, Abrahamsen B, Schwartz AV, Ferrari SL, et al. Mechanisms of diabetes mellitus-induced bone fragility. Nat Rev Endocrinol. 2017;13(4):208-19.

147. Bjørnerem Å, Ghasem-Zadeh A, Bui M, Wang X, Rantzau C, Nguyen TV, et al. Remodeling markers are associated with larger intracortical surface area but smaller trabecular surface area: a twin study. Bone. 2011;49(6):1125-30.

148. Nilsson AG, Sundh D, Johansson L, Nilsson M, Mellström D, Rudang R, et al. Type 2 Diabetes Mellitus Is Associated With Better Bone Microarchitecture But Lower Bone Material Strength and Poorer Physical Function in Elderly Women: A Population-Based Study. J Bone Miner Res. 2017;32(5):1062-71.

**Paper I**

# Appendix A

Questionnaire Pre-screening, Norwegian version

**Telefonscreening for case og kontroller i Beinstrukturstudien 01-2011.**

**Løpenr. KFS:** _____      **Case: 1001, 1002, 1003 osv.  Kontroller: 1, 2, 3 osv**

**Intervjuer:** _____      **Dato for telefonscreening:** _____

**Kort presentasjon, takke for svar på invitasjonen, litt informasjon om studien, noen spørsmål for å kartlegge om vedkommende kan delta i studien.**

**Bruker du medisiner mot beinskjørhet?**  ( Både case- og kontrollgruppen)
☐ **Ja**      **Hvis ja → Ekskluderes**
☐ **Nei**     **Hvis nei → Gå videre**

**Har du hatt brudd i lårhalsen, skuldra eller håndleddet etter at du fylte 50 år?** ( Både case- og kontrollgruppen)
☐ **Ja**      **Hvis ja → Gå videre til casegruppen**
☐ **Nei**     **Hvis nei → Gå videre for vurdering om de passer inn i kontrollgruppen**

**Har du noen gang hatt et brudd?**    (Kontrollgruppen)
☐ **Nei**     **Hvis nei → Gå videre i kontrollgruppen**
☐ **Ja**      **Hvis ja → Kontrollgruppen ekskluderes**

**Er det greit at din sykehusjournal v/ UNN sjekkes av studielege i forhold til brudd?** ( Både case- og kontrollgruppen)
☐ **Ja**
☐ **Nei**

**Har du hofteprotese eller innsatte skruer eller metall i hofte pga lårhalsbrudd?** ( Både case- og konrollgruppen)
☐ **Ja**      **Hvis ja → Ekskluderes**
☐ **Nei**     **Hvis nei → Gå videre**

**Merknadsfelt:** _____

**Konklusjon:**      ☐ **Kan delta i casegruppen**
                         ☐ **Kan delta i kontrollgruppen**
                         ☐ **Kan ikke delta**
                         ☐ **Tvil om kan delta**

**Hvis tvil om kan delta, send mail til Åshild Bjørnerem. "Knapp" for automatisk sending.**

**Svar fra Åshild Bjørnerem:**
☐ **Inkluderes i casegruppen.**
☐ **Inkluderes i kontrollgruppen**
☐ **Ekskluderes**

**Merknadsfelt:** _____

**Dato:** _____      **Signatur:** _____

# Appendix B

Questionnaire, Norwegian version

Dato ☐☐.☐☐.☐2☐0☐1☐    Løpe nr ☐☐☐☐

Initialer ☐☐☐

## HELSE, BRUDD OG SYKDOM

**Hvordan vurderer du din egen helse sånn i alminnelighet? (sett kun ett kryss)**

☐ Meget god

☐ God

☐ Verken god eller dårlig

☐ Dårlig

☐ Meget dårlig

**Har du hatt ett eller flere brudd etter at du fylte 50 år?**

☐ Ja    ☐ Nei

**Hvis JA, hvor mange brudd har du hatt?**

Antall brudd ☐☐

| Etter at du fylte 50 år, har du hatt | Alder første gang | Fikk du dette bruddet i forbindelse med en trafikkulykke? | Ble det tatt røntgenbilde i Tromsø? | Hvis bruddet skjedde utendørs, var det på is/snø? |
|---|---|---|---|---|
| lårhalsbrudd? ☐ Ja ☐ Nei | ☐☐ | ☐ Ja ☐ Nei | ☐ Ja ☐ Nei | ☐ Ja ☐ Nei |
| håndleddsbrudd? ☐ Ja ☐ Nei | ☐☐ | ☐ Ja ☐ Nei | ☐ Ja ☐ Nei | ☐ Ja ☐ Nei |
| skulderbrudd? ☐ Ja ☐ Nei | ☐☐ | ☐ Ja ☐ Nei | ☐ Ja ☐ Nei | ☐ Ja ☐ Nei |
| ankelbrudd? ☐ Ja ☐ Nei | ☐☐ | ☐ Ja ☐ Nei | ☐ Ja ☐ Nei | ☐ Ja ☐ Nei |
| annet brudd? ☐ Ja ☐ Nei | ☐☐ | ☐ Ja ☐ Nei | ☐ Ja ☐ Nei | ☐ Ja ☐ Nei |

**Har du falt i løpet av det siste året, fra høyde som ikke var over gulvnivå? (f. eks på gulv, vei, gate) (sett ett kryss)**

☐ Nei

☐ Ja, 1-2 ganger

☐ Ja, 2-5 ganger

☐ Ja, mer enn 5 ganger

**Hvis du har falt i løpet av den siste måneden, fra høyde som ikke var over gulvnivå (f. eks på gulv, vei, gate), hvor mange ganger har du falt siste måned ?**

Antall ganger falt ☐☐

**Har noen av dine foreldrene hatt lårhalsbrudd:**

☐ Mor    ☐ Far    ☐ Ingen

11061

Løpe nr ☐☐☐☐☐

| Har du eller har du hatt: | | Alder første gang |
|---|---|---|
| Beinskjørhet | ☐ Ja  ☐ Nei | |
| Diabetes / sukkersyke | ☐ Ja  ☐ Nei | |
| Hjerneslag / hjerneblødning | ☐ Ja  ☐ Nei | |
| Lavt stoffskifte | ☐ Ja  ☐ Nei | |
| Høyt stoffskifte | ☐ Ja  ☐ Nei | |
| Kreftsykdom | ☐ Ja  ☐ Nei | |
| Leddgikt (Rheumatoid artritt) | ☐ Ja  ☐ Nei | |
| Kronisk tarmsykdom (f.eks Ulcerøs kolitt eller Morbus Crohn) | ☐ Ja  ☐ Nei | |

Har du / har du hatt andre kroniske tilstander / sykdommer, eventuelt nevn kort hvilke?

..............................................................................................................................................

..............................................................................................................................................

## RØYKING OG ALKOHOL

**Har du røykt/røyker du daglig?**

☐ Ja, nå

☐ Ja, tidligere

☐ Aldri

**Hvor mange år til sammen har du røykt daglig?**

Antall år ☐☐

**Hvor mange sigaretter røykte /røyker du vanligvis daglig?**  Antall sigaretter ☐☐

**Hvor ofte drikker du alkohol?**

☐ Aldri

☐ Månedlig eller sjeldnere

☐ 2-4 ganger pr. måned

☐ 2-3 ganger pr. uke

☐ 4 eller flere ganger pr. uke

**Hvor mange enheter alkohol (en øl, et glass vin, eller en drink) tar du vanligvis når du drikker?**

☐ 1-2

☐ 3-4

☐ 5-6

☐ 7-9

☐ 10 eller flere

11061

Løpe nr

## BRUK AV MEDISINER

| Bruker du eller har du brukt | Ja | Før | Aldri | Alder første gang | Alder da du sluttet | Brukt hvor mange år |
|---|---|---|---|---|---|---|
| Hormonbehandling mot plager i overgangsalder (tabletter eller plaster) (vi mener ikke Ovesterin, Oestriol) | ☐ | ☐ | ☐ | | | |
| Femar eller Arimidex tabletter for behandling av brystkreft | ☐ | ☐ | ☐ | | | |
| Kortikosteroider (Prednisolon tabletter) | ☐ | ☐ | ☐ | | | |
| Vanndrivende eller annen medisin mot høyt blodtrykk | ☐ | ☐ | ☐ | | | |
| Kolesterolsenkende medisin | ☐ | ☐ | ☐ | | | |
| Sovemedisin eller beroligende medisin | ☐ | ☐ | ☐ | | | |
| Insulin | ☐ | ☐ | ☐ | | | |
| Tabletter mot sukkersyke | ☐ | ☐ | ☐ | | | |
| Kalktabletter | ☐ | ☐ | ☐ | | | |
| Tran, trankapsler eller andre vitamintabletter | ☐ | ☐ | ☐ | | | |

Skriv ned navn på alle medisinene du bruker (bruk eventuelt eget ark)

..............................................................................

..............................................................................

..............................................................................

..............................................................................

## FØDSLER OG AMMING

**Hvor mange barn har du født?**

Antall barn ☐☐

**Hvis du har født barn, hvor mange måneder ammet du dem til sammen?**

Antall måneder med amming ☐☐

## MENSTRUASJON

**Hvor gammel var du da du fikk menstruasjonen første gang?**   Alder ☐☐

**Hvor gammel var du da menstruasjonen stoppet?**   Alder ☐☐

**Hvorfor stoppet menstruasjonen? (sett ett kryss)**

☐ Den stoppet av seg selv

☐ Operasjon på livmoren

☐ Operert bort begge eggstokkene

☐ Operert bort begge eggstokkene og livmoren

☐ Strålebehandling/cellegift

11061

Løpe nr

# FYSISK AKTIVITET

## Hvis du er i lønnet eller ulønnet arbeid, hvordan vil du best beskrive arbeidet ditt? (sett ett kryss)

☐ for det meste stillesittende arbeid (f.eks skrivebordsarbeid, montering)

☐ arbeid som krever at du går mye ( f. eks ekspeditørarbeid, lett industriarbeid, undervisning)

☐ arbeid der du går og løfter mye ( f.eks postbud, pleier, bygningsarbeider)

☐ tungt kroppsarbeid (f. eks skogsarbeid, tungt jordbruksarbeid, tungt bygningsarbeid)

## Angi bevegelse og kroppslig anstrengelse i din fritid. Hvis aktiviteten varierer meget f.eks mellom sommer og vinter, så ta et gjennomsnitt. Spørsmålet gjelder bare det siste året (sett kryss i den ruta som passer best)

☐ leser, ser på fjernsyn eller annen stillesittende beskjeftigelse

☐ spaserer, sykler eller beveger deg på annen måte minst 4 timer i uken (her skal du også regne med gang eller sykling til arbeidsstedet, søndagsturer med mer)

☐ driver mosjonsidrett, tyngre hagearbeid, snømåking eller lignende (aktiviteten skal vare minst 4 timer i uka)

☐ trener hardt eller driver konkurranseidrett regelmessig og flere ganger i uka

## Hvis du driver idrett eller mosjon, hvilken idrett eller aktivitet driver du på med?

...................................................................................................................

## Hvor ofte driver du mosjon? (med mosjon mener vi at du f.eks går en tur, går på ski, svømmer eller driver trening/idrett).

☐ Aldri

☐ Sjeldnere enn en gang i uka

☐ En gang i uka

☐ 2-3 ganger i uken

☐ omtrent hver dag

## Hvor hardt mosjonerer du i gjennomsnitt? (sett ett kryss)

☐ Tar det rolig uten å bli andpusten eller svett

☐ Tar det så hardt at jeg blir andpusten og svett

☐ Tar meg nesten helt ut

## Hvor lenge holder du på hver gang i gjennomsnitt? (sett ett kryss)

☐ mindre enn 15 minutter

☐ 15-29 minutter

☐ 30 minutter - 1 time

☐ mer enn 1 time

Dine kommentarer til spørreskjema

...................................................................................

...................................................................................

...................................................................................

...................................................................................

**Takk for hjelpen!**

11061