UiT
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Faculty of Health Sciences

Department of Clinical Medicine

Cortical bone and fracture risk: The Tromsø Study

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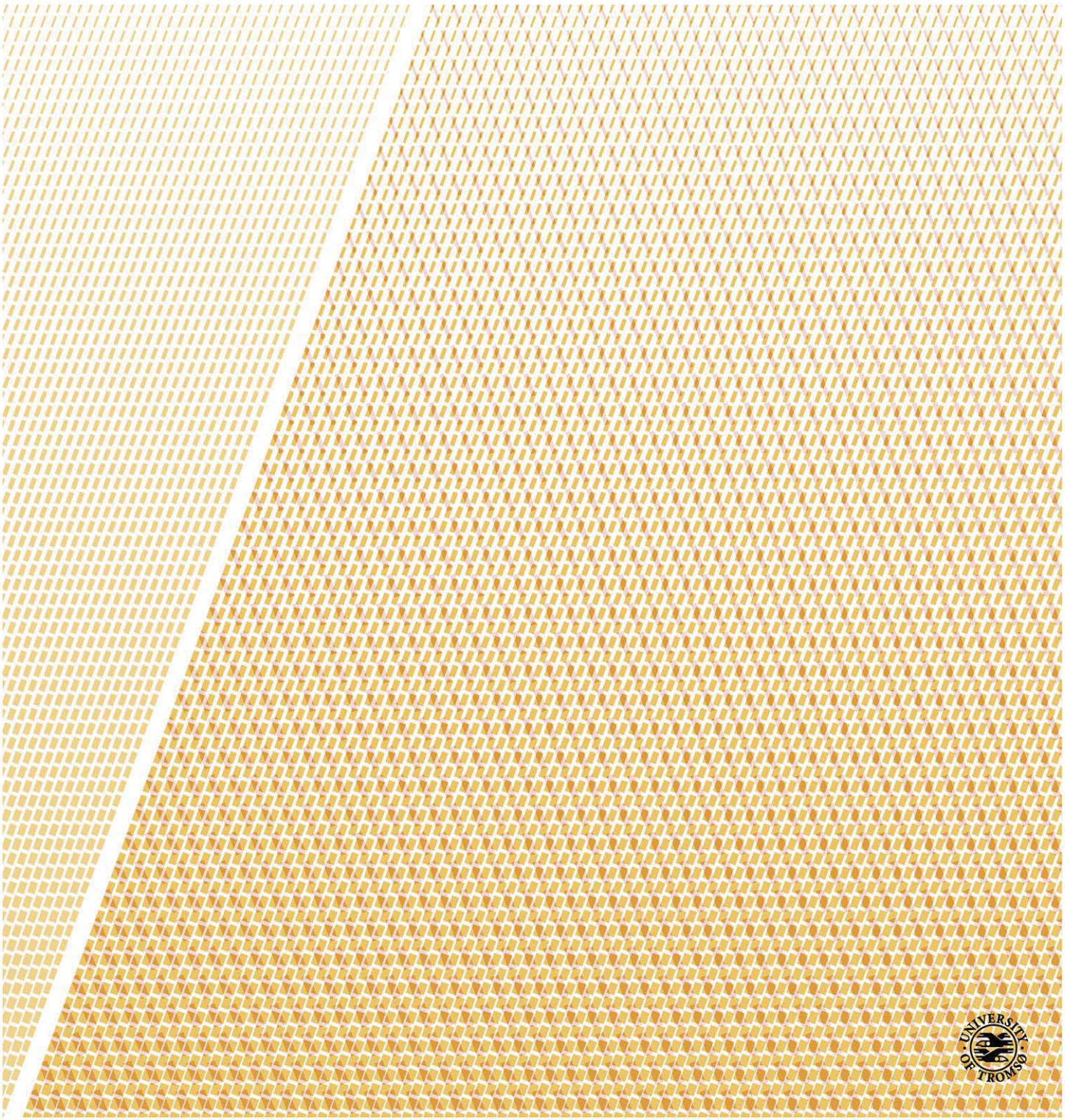
Rita Kral

A dissertation for the degree of Philosophiae Doctor – November 2017



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Contents **5**
Acknowledgement **6**
Summary **7**
List of papers **9**
Abbreviations **10**
1 Background **11**
1.1 Epidemiology and burdens of fragility fracture 11
1.2 Risk factors of fracture 11
1.3 Fracture risk assessment 12
1.4 Bone architecture and physiology 14
1.4.1 Composition of bone 14
1.4.2 Architecture of bone: cortical and trabecular bone 15
1.4.3 Bone remodeling, repair, and renewal and physiology of bone loss 17
2 Rationale and aims of the thesis **21**
3 Materials and Methods **23**
3.1 Study population 23
3.2 Ethics 25
3.3 Data from questionnaires and measurements 25
3.4 FRAX and Garvan estimates 26
3.5 Quantification and validation of bone architecture 29
3.6 Statistical analysis 34
4 Main results **36**
5 Discussion **39**
5.1 Methodological considerations 39
5.1.1 Internal validity 39
5.1.2 External validity or generalizability 43
5.2 Significance of the results 44
5.2.1 FRAX, Garvan and cortical porosity 44
5.2.2 Patient profile in fracture cases identified by high cortical porosity 45
5.2.3 Type 2 diabetes mellitus and cortical porosity 46
6 Conclusions, implications, future perspectives **48**
6.1 Conclusions 48
6.2 Implications and further research 49
References **50**
Paper I-III
Appendices

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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 ≥ 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 ≥ 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 ≥ 3.
[e]Number of falls: 0, 1, 2, or ≥ 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).

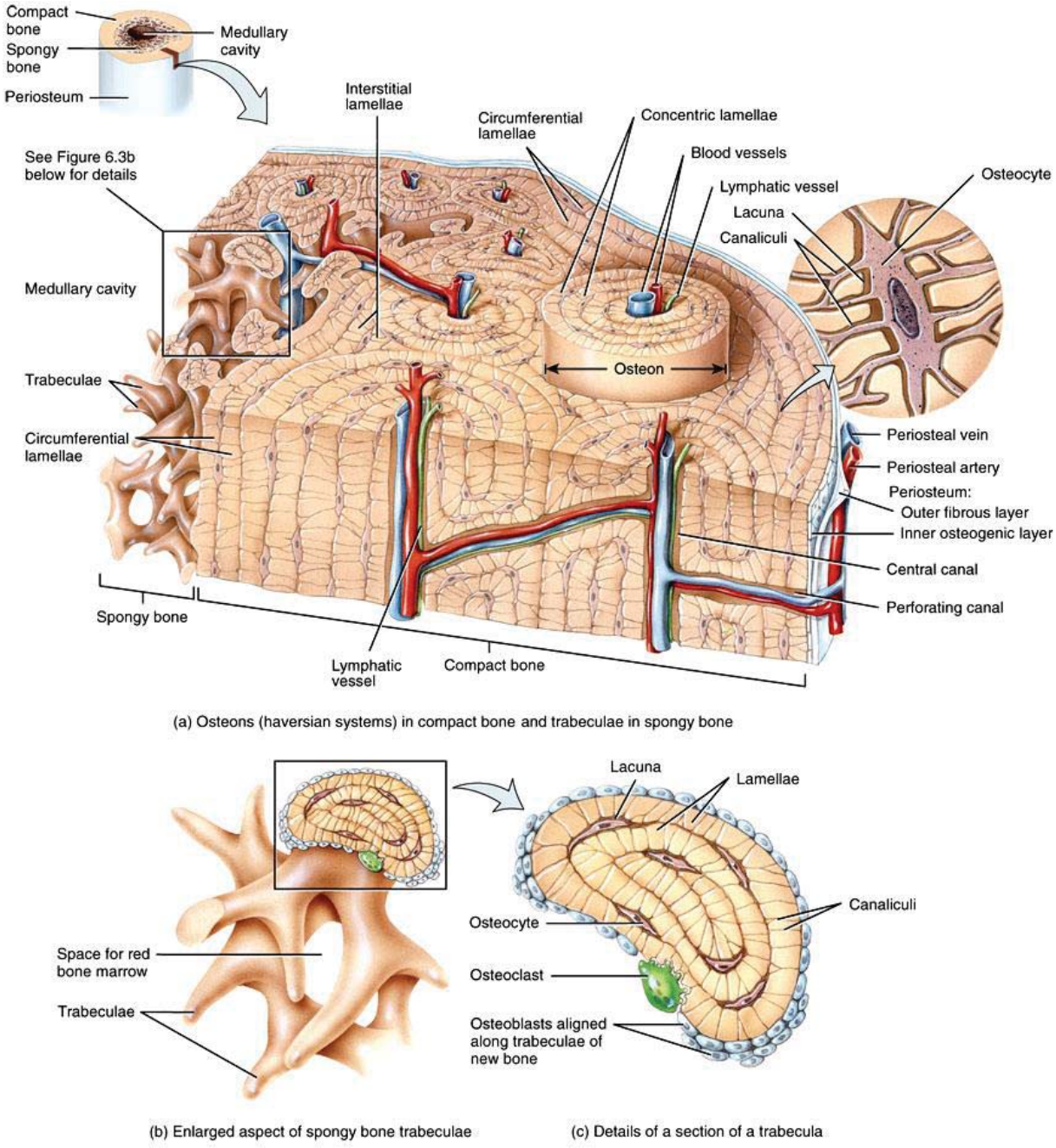

Figure 06.03 Tortora - PAP 12/e

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).

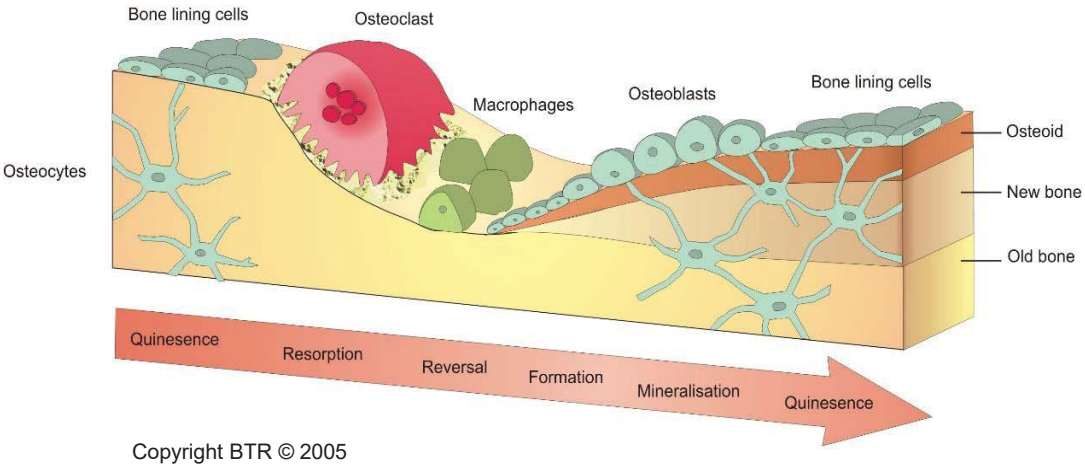

Fig. 2. The bone remodeling process. Bone is continuously remodeled at discrete sites in the skeleton in order to maintain the integrity of the tissue. During this process, old bone is resorbed by osteoclasts and replaced with new osteoid, secreted by osteoblasts. First, osteoclasts are activated, and 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).

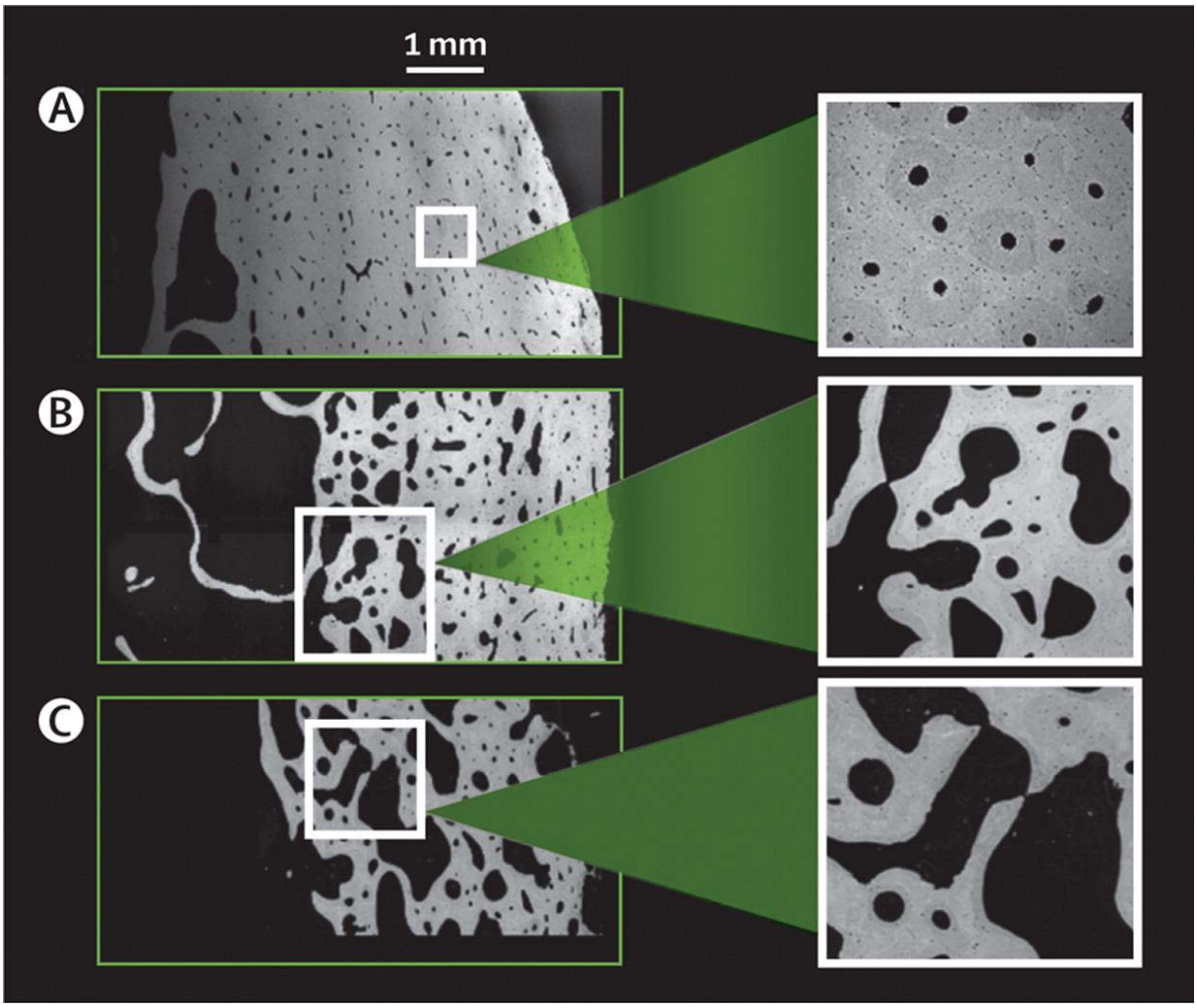

Fig. 3. Porosity in post-mortem specimens from three women of different ages
(A) Micrograph of a specimen from a 29-year-old woman. Pores are regular in shape and evenly distributed in the cortex. (B) Micrograph of a specimen from a 67-year-old woman. Pores are large, irregularly shaped, and have coalesced 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).

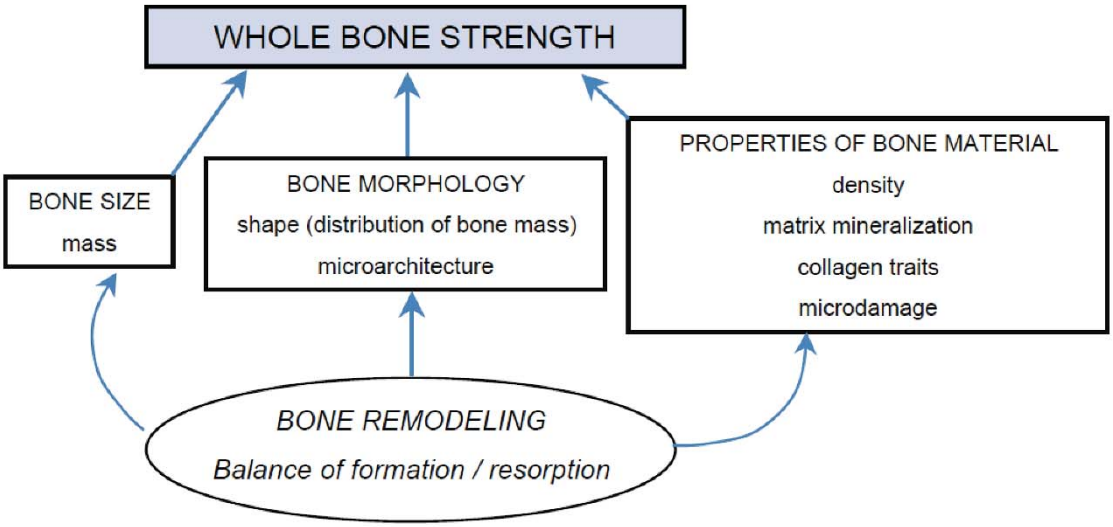

Fig. 4. Determinants of whole bone strength
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2 Rationale and aims of the thesis

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.

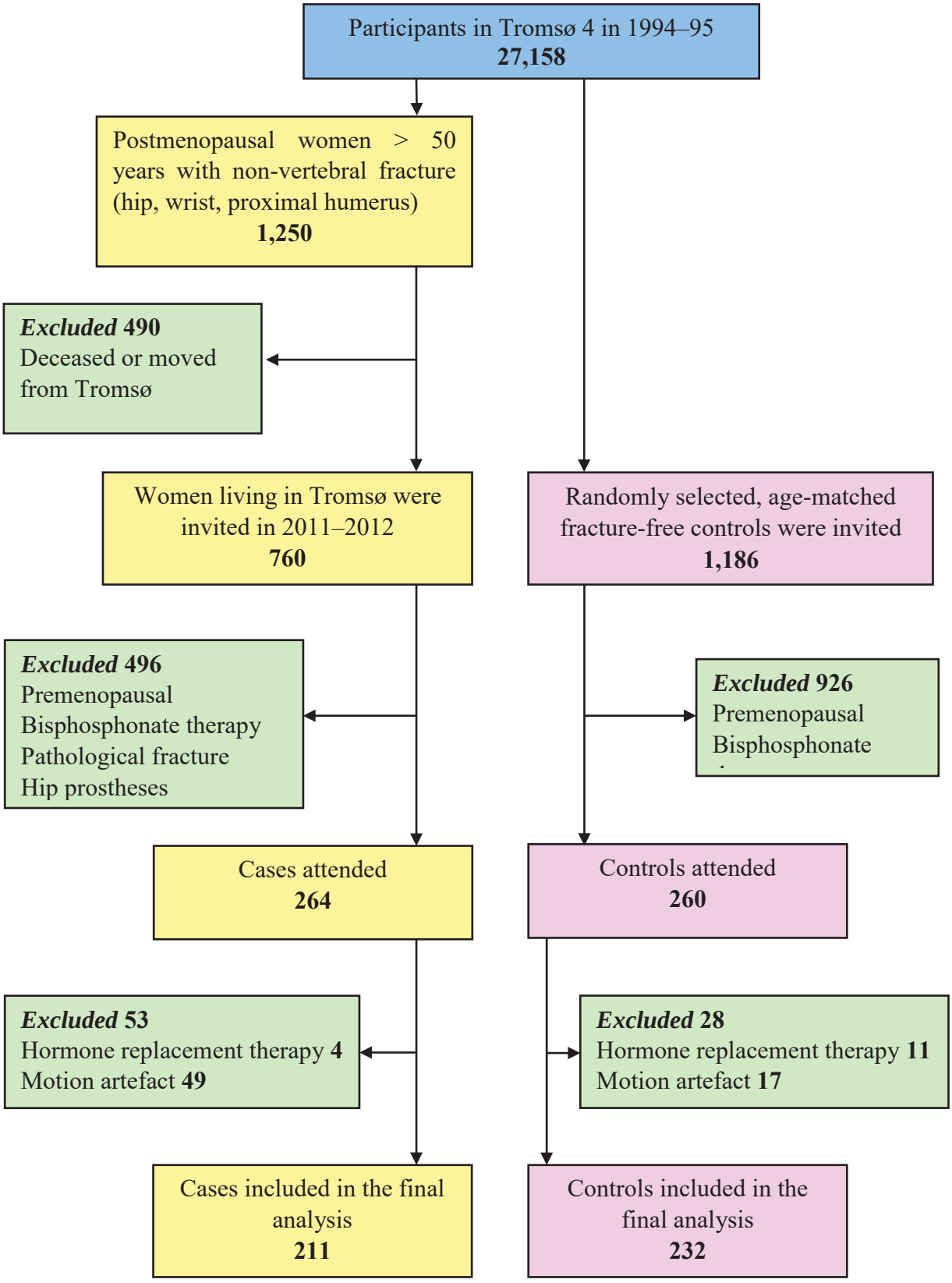


Fig. 5. Participants in this nested case-control study based on the Tromsø Study in 1994–95

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 ± 1.2 vs. 68.3 ± 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 ± 2.2 vs. 68.2 ± 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/height^2. 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

	Medical records		
Self-reported	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

	Based on medical records						
Self-reported	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

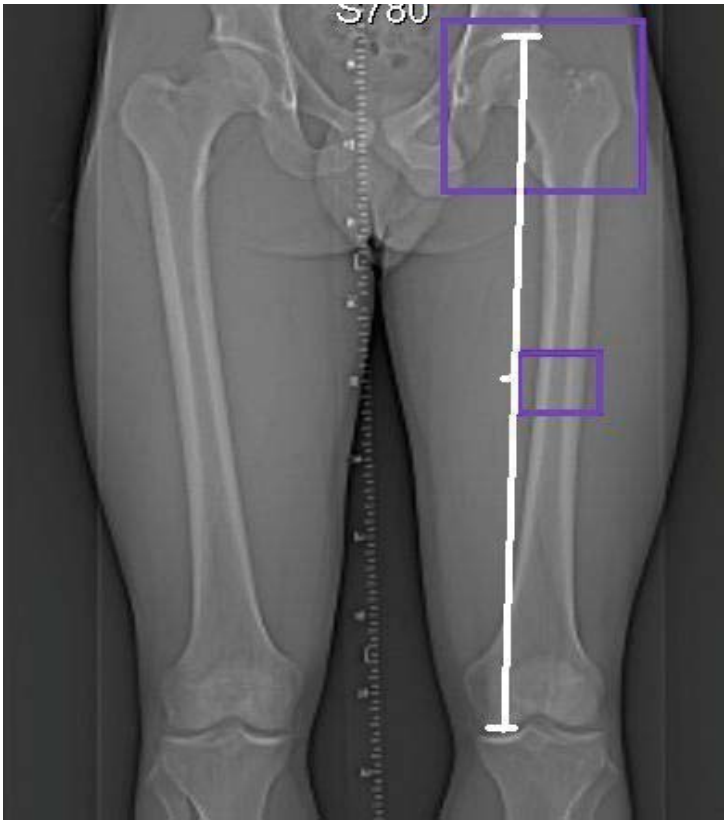
This validation process identified 61 women with previous or subsequent fractures through medical records. Each of these 61 women had a total of two or more fractures (**Table 5**). We included this information in the latest calculation of their FRAX and Garvan estimates. The updated FRAX estimates changed 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).

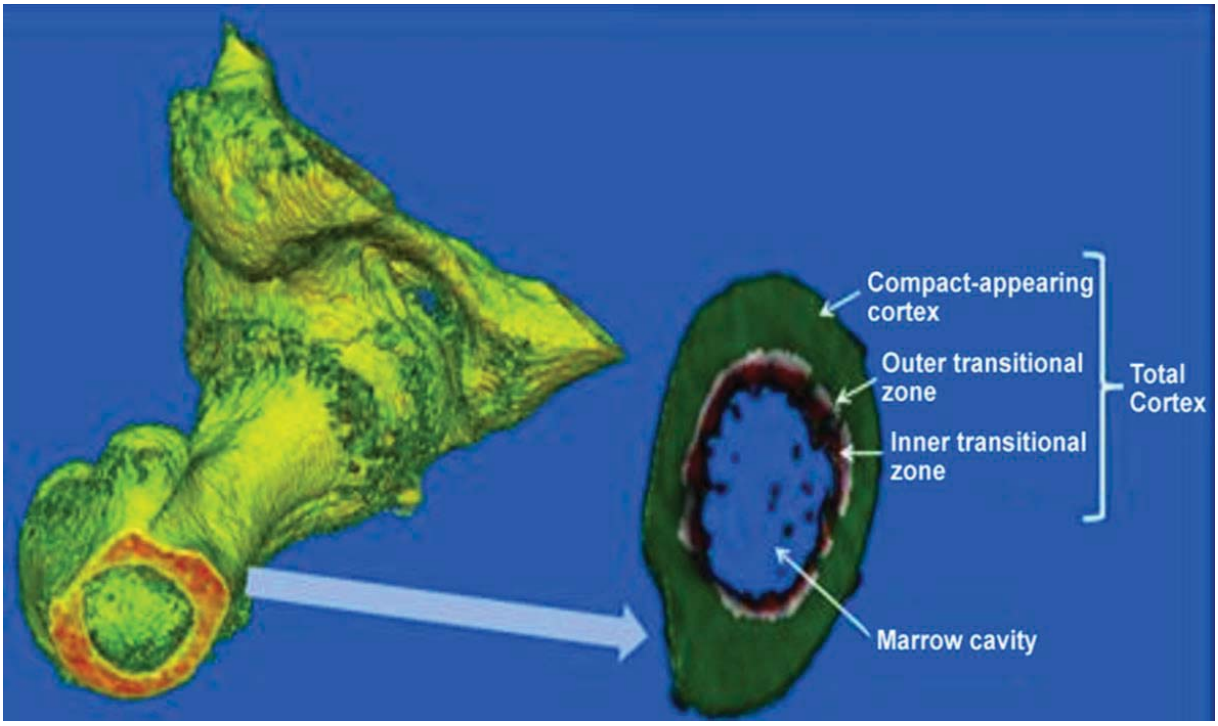


Fig. 7. Cross-section image of proximal femur and its compartments
Segmented computed tomography image obtained at the proximal femur using StrAx1.0, a non-threshold-based segmentation algorithm, showing the total cortex (the area used for the cortical porosity measurements), consisting of the three 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).

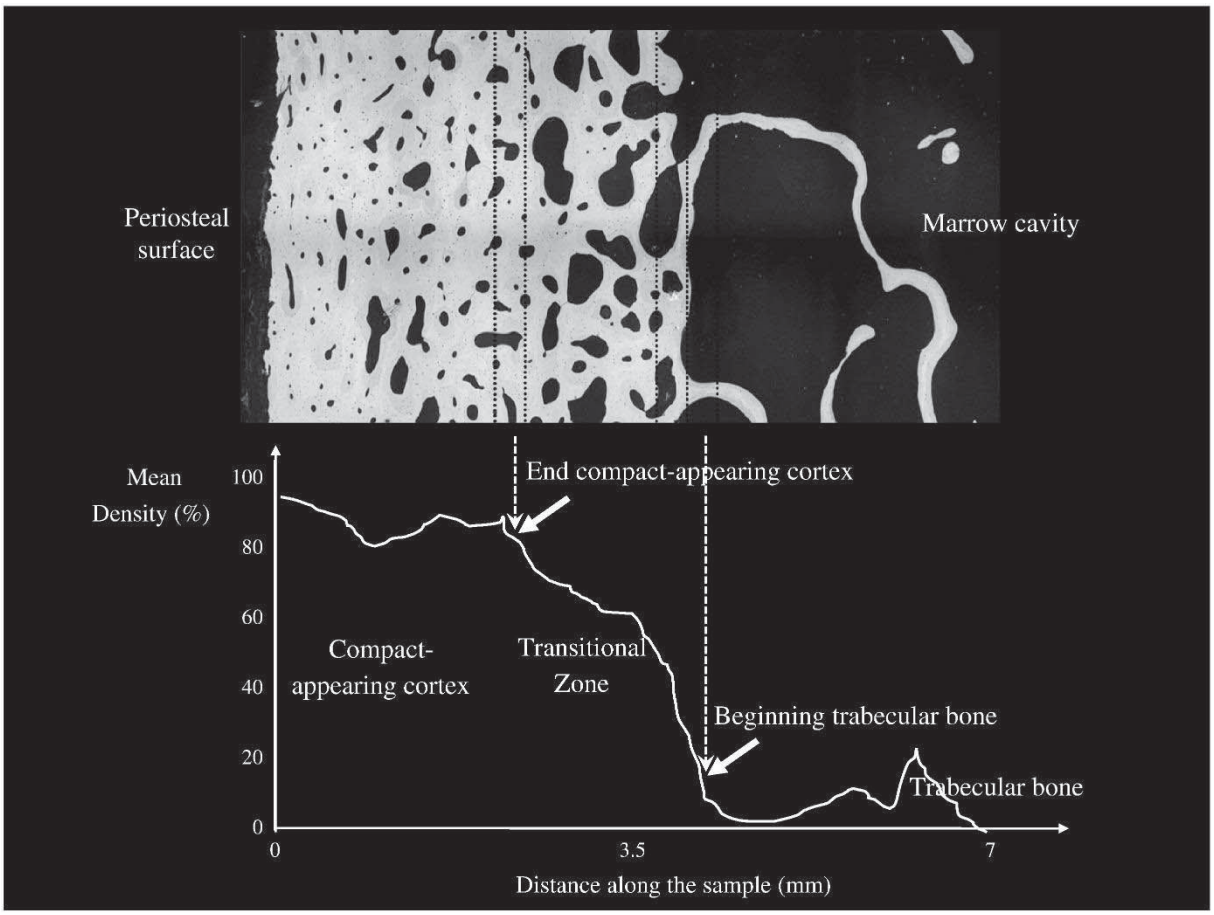


Fig. 8. A scanning electron microscopic image of the subtrochanteric showing the compact-appearing cortex, transitional zone, and trabecular compartment. The density profile curve produced has two plateaus; one corresponding to the compact-appearing cortex and one corresponding to the trabecular compartment. 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).

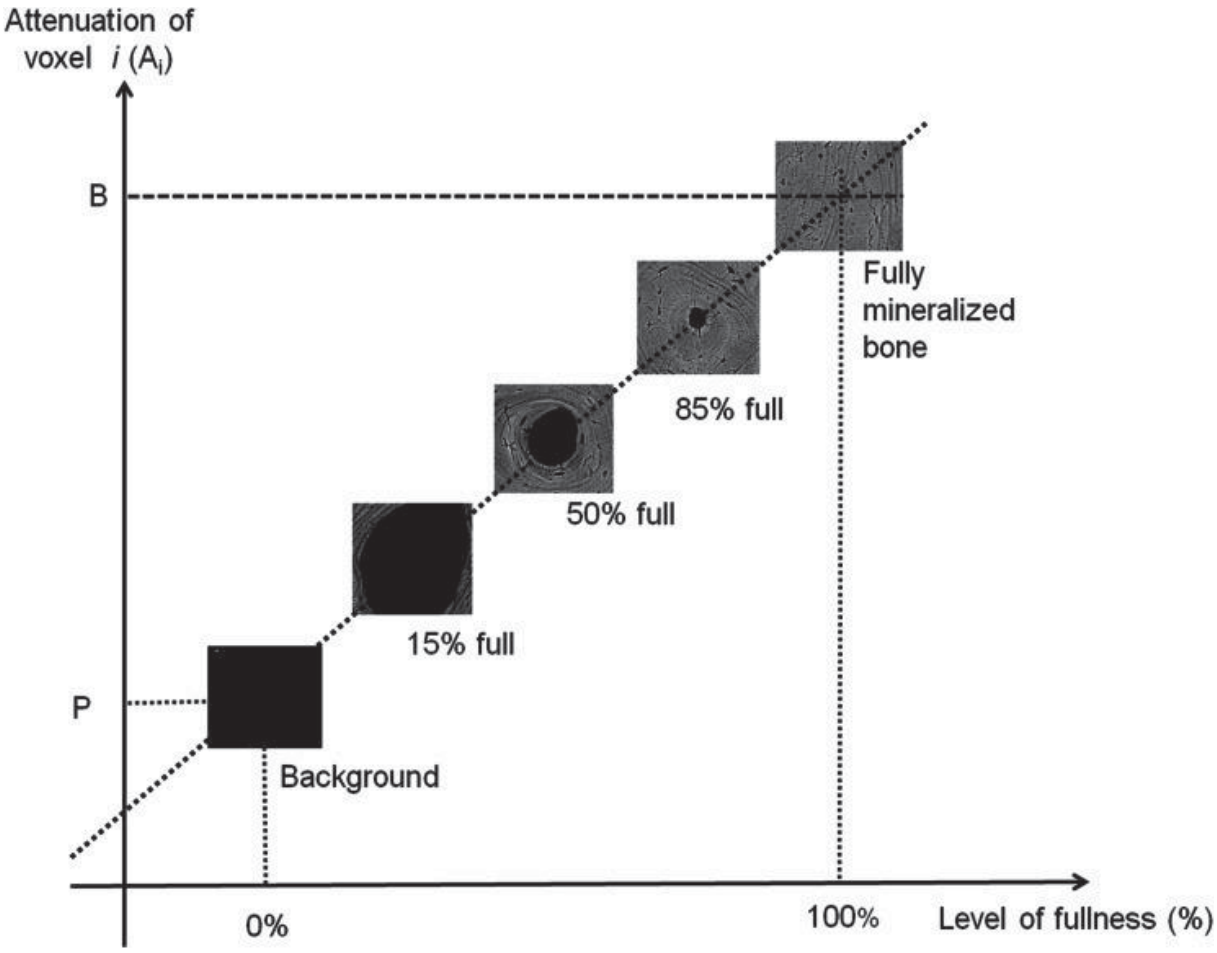


Fig. 9. To measure porosity, two referent attenuation values are required P: the background (muscle, water etc.) and B: the fully mineralized bone matrix (1200 mg HA/cm^3). The proportion of the voxel volume occupied by mineralized bone matrix is its level of fullness (LOF) and is estimated as (LOF) %=(Ai-P)/(B-P), where Ai is the attenuation of voxel i. From LOF, the void volume of 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 75^{th}, 80^{th}, and 90^{th} percentile, and cortical thickness below the 10^{th}, 20^{th}, and 25^{th} 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 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 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 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 (> 80^{th} percentile) or cortical thickness (< 20^{th} 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 (mm^2)	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.

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Paper I

PLOS ONE

RESEARCH ARTICLE

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

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Abstract

The Fracture Risk Assessment Tool (FRAX) and Garvan Calculator have improved the individual prediction of fracture risk. However, additional bone measurements that might enhance the predictive ability of these tools are the subject of research. There is increasing interest in cortical parameters, especially cortical porosity. Neither FRAX nor Garvan include measurements of cortical architecture, important for bone strength, and providing independent information beyond the conventional approaches. We tested the hypothesis that cortical parameters are associated with fracture risk, independent of FRAX and Garvan estimates. This nested case-control study included 211 postmenopausal women aged 54–94 years with nonvertebral fractures, and 232 controls from the Tromsø Study in Norway. We assessed FRAX and Garvan 10-year risk estimates for fragility fracture, and quantified femoral subtrochanteric cortical porosity, thickness, and area from computed tomography images using StrAx1.0 software. Per standard deviation higher cortical porosity, thinner cortices, and smaller cortical area, the odds ratio (95% confidence interval) for fracture was 1.71 (1.38–2.11), 1.79 (1.44–2.23), and 1.52 (1.19–1.95), respectively. Cortical porosity and thickness, but not area, remained associated with fracture when adjusted for FRAX and Garvan estimates. Adding cortical porosity and thickness to FRAX or Garvan resulted in greater area under the receiver operating characteristic curves. When using cortical porosity (>80th percentile) or cortical thickness (<20th percentile) combined with FRAX (threshold >20%), 45.5% and 42.7% of fracture cases were identified, respectively. Using the same cutoffs for cortical porosity or thickness combined with Garvan (threshold >25%), 51.2%

interested and qualified researchers after application and agreement with the Department of Community Medicine (UiT The Arctic University of Norway) according to their data application process. In addition to the application, an existing or new approval from the Regional Committee for Medical Research Ethics (REK) is required. Guidelines on how to access the data are available at the website: http://tromsoundersokelsen.uit.no/tromso/ and information on how to apply for data access can be found at https://en.uit.no/forskning/forskningsgrupper/sub?p_document_id=453582&sub_id=71247. All enquiries about the Tromsø Study should be sent by e-mail to: tromsous@ism.uit.no, or Kristin Sørensen may be contacted by phone at +47 776 45348.

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and 48.3% were identified, respectively. Specificity for all combinations ranged from 81.0–83.6%. Measurement of cortical porosity or thickness 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. In conclusion, cortical parameters may help to improve identification of women at risk for fracture.

Introduction

Fragility fracture is a growing health problem due to a longer lifespan and an aging population [1,2]. Therefore, it is important to identify individuals at high fracture risk, and offer them appropriate care and treatment. The most widely used measurement to assess fracture risk is areal bone mineral density (aBMD) [3–7]. However, aBMD alone has low sensitivity for fracture [3], as most of the fragility fractures occur in individuals with an aBMD in the osteopenic or normal range, and not in those with an aBMD below the osteoporosis threshold [4]. In order to address this lack of sensitivity, tools such as the Fracture Risk Assessment Tool (FRAX) [5,6] and the Garvan Fracture Risk Calculator have been developed [7].

The FRAX tool is widely used to calculate the 10-year probability of hip and major osteoporotic fracture (hip, proximal humerus, wrist, and clinical spine) based on the individual's risk factor profile [5,6]. FRAX includes age, sex, body mass index (BMI) computed from height and weight, and clinical risk factors such as a prior fragility fracture, parental history of hip fracture, current smoking, alcohol consumption, oral glucocorticoid use, rheumatoid arthritis, other causes of secondary osteoporosis, and femoral neck (FN) aBMD. The Garvan Fracture Risk Calculator is a simpler tool [7,8], and only includes five risk factors: age, sex, number of fractures since an age of 50 years, number of falls over the last 12 months, and FN aBMD (or body weight). Garvan estimates the individual's 5-year and 10-year absolute risk for hip fracture and any fragility fracture (hip, humerus, wrist, metacarpal, scapula, clavicle, sternum, pelvis, distal femur, proximal tibia, patella, spine [symptomatic]) [7]. Both FRAX and Garvan tools can be used with or without FN aBMD.

Additional skeletal determinants of bone strength are subject to clinical research, which may modify or enhance the predictive ability of existing tools. The FRAX estimates can be adjusted for trabecular bone score (TBS), which is an index of trabecular microarchitecture [9,10]. However, both trabecular and cortical architecture are important for bone strength [9–12], but neither FRAX nor Garvan take cortical bone architecture into account, which is particularly important for bone strength as 80% of the skeleton consists of cortical bone [11,12]. There is increasing interest in measurements of cortical parameters, which may provide independent information regarding skeletal strength and fracture risk beyond these conventional approaches.

In a prospective study, cortical area and cortical bone mass of the distal tibia, but not cortical porosity, were associated with incident fractures, independent of FN aBMD and FRAX score, in older men [13]. In contrast, reports from cross-sectional studies have suggested that cortical porosity is associated with prevalent fracture in women and men [12,14–16]. Women with fractures have higher cortical porosity and thinner cortices than controls as shown in biopsies [17] and computed tomography (CT) scans of the proximal femur [14,18,19]. Moreover, cortical porosity is associated with fracture, independent of FRAX [12,18].

Although a measurement of cortical porosity combined with FRAX identified additional women with fracture than using FRAX alone, more than half of the fracture cases were still not

identified using either FRAX or cortical porosity [14]. Improving identification of individuals at high fracture risk is still a challenge. To the best of our knowledge, there is no study of the performance of cortical parameters independent of Garvan estimates. We aimed to explore this further by including cortical thickness and cortical area in the current analysis, and test whether combinations of cortical parameters with FRAX or Garvan estimates can provide additional information and improve identification of women with fracture beyond the existing tools. Therefore, this study tested the hypothesis that measurements of cortical parameters (porosity, thickness, and area) are associated with fracture risk, independent of FRAX or Garvan estimates.

Subjects and methods

Subjects

The Tromsø Study is a single-center, population-based study in Northern Norway, which conducted six surveys between 1974 and 2008 [20]. During the Tromsø 4 survey in 1994–95, 37,558 eligible inhabitants in Tromsø over 24 years old were invited to participate, and 27,158 (72%) agreed. Within these participants, all nonvertebral fractures that occurred between January 1, 1994 and January 1, 2010 were registered from the University Hospital of North Norway, Tromsø x-ray archives [21]. 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 and identified 1,250 women from the x-ray-based fracture registry that suffered at least one fracture of the hip, wrist, or proximal humerus after the age of 50 years [14,18,19]. We invited all 760 women who were still alive and living in Tromsø. All women who were willing to participate had a pre-screening phone call to determine whether they were eligible for participation in accordance with the inclusion and exclusion criteria. Those who were premenopausal, received bisphosphonates, or had hip prostheses or metal screws in the hip region were excluded the study. Since metal on one side of the hip can create noise in the CT images on both sides, many women with a hip fracture could not be included unless they had the metal removed. After screening, 264 fracture cases were included in this study [14,18,19]. Age-matched, fracture-free women, who were within the same 5-year age group, were randomly selected from the Tromsø 4 participants and 1186 were invited. After a pre-screening phone call to determine whether they were eligible and fracture-free, 260 controls were included. Of the total 524 participants, we excluded 15 women who were currently receiving hormone replacement therapy and 66 women due to movement artifacts during CT scanning. This resulted in 443 women in the final analyses: 232 controls and 211 fracture cases (4 hip, 181 wrist, and 26 proximal humerus). The median time since their index fracture was 6.6 y (range: 1–25 y). All variables included in the analysis were based on information obtained at the time of study enrollment between November 2011 and January 2013. All participants provided written informed consent. 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.

Methods

Variables and measurements. At enrollment of the study, the participants filled in a questionnaire that included information concerning all fractures after the age of 50 years (number and type of fracture), number of falls in the last year, diseases, use of medication, and lifestyle. Height and weight were measured while wearing light clothing and without shoes. BMI was calculated as weight/height^2. FN aBMD was measured using dual-energy x-ray

absorptiometry (GE Lunar Prodigy, Lunar Corporation, Madison, WI, USA) and the coefficient of variation was 1.7%.

We entered the 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. The index fractures used as the inclusion criteria for this study were not included as a "previous fracture" in the calculation of the FRAX estimate, because the aim was to assess 10-year probability of fracture before the event, not the probability of fracture after this event [12, 14]. The index fractures were not included in the number of fractures in the Garvan estimate.

However, the "previous fractures" (before the index fracture) and "subsequent fractures" (after the index fracture) should both be used equally in the calculation of FRAX and Garvan estimates. Therefore, we validated these fractures through the medical records of 91 women, who either had a self-reported "previous fracture" (n = 54), a total of two or more self-reported fractures (n = 71), or both (n = 34). The validation confirmed that 61 of 91 women had a previous or subsequent fracture, which we included in the calculation of their FRAX estimates. The same 61 women had one or more fractures, which we included in the calculation of their Garvan estimates.

CT scans (Siemens Somatom Sensation 16, Erlangen, Germany) of the non-dominant hip were performed at the Department of Radiology in the University Hospital of North Norway [14]. 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 [14]. 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 [22]. Quality control was carried out by scanning a phantom containing rods of hydroxyapatite (QRM Quality Assurance in Radiology and Medicine GmbH, Moehrendorf, Germany). The CT images were sent to Melbourne, Australia, and analyzed by collaborators, who were blinded to the fracture status, using the StrAx1.0 software (StraxCorp Pty Ltd, Melbourne, Australia). 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 with thicker cortices, which started at the tip of the lesser trochanter as shown previously [14,23].

The StrAx1.0 software is a non-thresholding method that automatically segments the bone within the region of interest into its compartments: compact cortex and outer and inner transitional zones (TZ) [14,23]. This was performed similarly in low-resolution images [14,23] as in high-resolution images [24]. Of the total cortex at this subtrochanteric site, 70.0% was compact cortex, while 22.3% and 11.7% were outer and inner TZ, respectively. Porosity within the total cortex and each cortical compartment was quantified automatically throughout the region of interest using the StrAx1.0 software [14,23,24] and coefficient of variation was 0.3–2.3% [14,23]. The agreement (R^2) between CT and high-resolution peripheral quantitative computed tomography (HR-pQCT) ranged from 0.86 to 0.96 for quantification of porosity at the same femoral subtrochanteric site [14,23]. The correlation between porosity (ranged from 40 to 95%), quantified using CT and HR-pQCT, was linear [23].

The porosity quantified by this algorithm is the proportion of emptiness within each voxel or the fraction of the bone occupied by void [24, 25]. StrAx1.0 quantifies porosity in low-resolution images, and similarly for high-resolution images, even though pores are not visible. It is a density-based, indirect measure of porosity, and the size and number of pores are not determined [14,18,19,23,25]. StrAx1.0 software quantifies porosity as a fraction of void, regardless

of size of the pores, and indirectly captures porosity produced by large and small pores. It accounts for partial volume effect by including not only void within completely empty voxels, but also partly empty voxels [24]. By using the StrAx1.0 software, we can quantify porosity of the compact cortex and the TZ. It is thus more inclusive than traditional measurements, and the porosity is higher than what has been previously reported using other methods [24,25].

Statistical analyses. Age-adjusted analysis of variance was used to compare cases and controls. Logistic regression analysis was used to calculate odds ratio (OR) for fracture with 95% confidence interval (CI) adjusted for age, height, weight, and FN aBMD, or adjusted for FRAX or Garvan estimates. Due to 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 selection of thresholds for each of the variables and for further analysis of combinations of variables. We further calculated the net reclassification improvement (NRI) to quantify how well the new models correctly reclassified women [26]. 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 net proportion of women reclassified correctly were calculated from the number of women with and without events reclassified correctly or incorrectly. 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 who was exposed. Assuming a power of 80%, and a significance level of 5%, we would be able to detect an OR = 2.0 with 165 fracture cases and 165 controls (1:1), OR = 1.8 with 230 cases and 230 controls (1:1), and OR = 1.6 with 363 cases and 363 controls (1:1), if 25% of the sample was exposed to high porosity. Analyses were performed using SAS Software package, v9.4 (SAS Institute Inc., Cary, NC, USA) and $p < 0.050$ was considered significant.

Results

FRAX, Garvan, and cortical bone parameters in cases and controls. Women with non-vertebral fracture were taller, had lower BMI, lower FN aBMD, and higher FRAX and Garvan estimates than age-matched, fracture-free controls ($p < 0.050$ for all; Table 1). Cases had higher cortical porosity, thinner cortices, and smaller cortical area at the femoral subtrochanter ($p < 0.050$ for all). There was no difference between cases and controls in self-reported health, weekly hours of physical activity, and number of falls during the last year.

FRAX, Garvan, cortical parameters, and odds for fracture. Each standard deviation higher for FRAX and Garvan estimates increased the odds for fracture; OR (95% CI) were 2.04 (1.64–2.53) and 2.31 (1.84–2.91), respectively (Table 2). Each standard deviation higher for cortical porosity, thinner cortices, and smaller cortical cross-sectional area at the femoral subtrochanter increased odds for fracture (1.71 [1.38–2.11], 1.79 [1.44–2.23], and 1.52 [1.19–1.95], respectively). We explored each component of the FRAX and Garvan tools. Early menopause and hypothyroidism were associated with increased odds for fracture, independent of age, height, and weight (1.81 [1.01–3.23] and 2.43 [1.36–4.34], respectively). Women with one or more falls within the last 12 months had no increased odds for fracture than those without falls (0.92 [0.62–1.36]).

Table 1. Characteristics of postmenopausal women by fracture status.

	Cases	Controls	p-value
n	**211**	**232**	
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/m^2)	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)	77.4 ± 16.8	77.8 ± 14.9	0.584
eGFR below 60 ml/min, n (%)	25 (11.9)	22 (9.5)	0.409
Femoral neck (FN) aBMD (mg/cm^2)	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/cm^3)	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 (mm^2)	409 ± 39.1	417 ± 39.4	0.029
Cortical vBMD (mg HA/cm^3)	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 are mean ± standard deviation or number (%).*Total number of fracture did not include index fractures. Cases and controls were compared using analysis of variance adjusted for age.
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 the 10-year probability of major fracture; Garvan, Fracture Risk estimate of the 10-year fracture risk for any fragility fracture.

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Table 2. Odds ratio (OR) and 95% confidence interval (CI) for non-vertebral fracture for each of the risk factors included in FRAX or Garvan estimates, and for the femoral subtrochanter architecture.

	SD unit	OR (95% CI)	p-values
Age	+ 7.21 year	1.13 (0.92–1.39)	0.242
Height	+ 6.40 cm	1.39 (1.12–1.72)	0.003
Weight	– 10.7 kg	1.19 (0.98–1.46)	0.085
Currently smoker	yes vs no	1.41 (0.78–2.56)	0.261
Parental hip fracture	yes vs no	0.97 (0.58–1.62)	0.892
Glucocorticoid use	yes vs no	5.08 (1.03–25.2)	0.047
Rheumatoid arthritis	yes vs no	1.95 (0.75–5.06)	0.170
Hyperthyroidism	yes vs no	1.63 (0.55–4.85)	0.383
Hypothyroidism	yes vs no	2.43 (1.36–4.34)	0.003
Ulcerative colitis/Crohn's disease	yes vs no	2.81 (0.96–1.04)	0.060
Diabetes	yes vs no	0.46 (0.08–0.77)	0.774
Early menopause < 45 year	vs ≥ 45 year	1.81 (1.01–3.23)	0.045
Femoral neck (FN) aBMD	– 0.111 mg/cm^2	2.11 (1.66–2.68)	< 0.001
FRAX estimate (%)	+ 6.82%	2.04 (1.64–2.53)	< 0.001
Falls in the last 12 months	≥1 vs 0	0.92 (0.62–1.36)	0.675
Garvan estimate (%)	+ 12.6%	2.31 (1.84–2.91)	< 0.001
Femoral subtrochanter architecture			
Cortical porosity	+ 4.01%	1.71 (1.38–2.11)	< 0.001
Cortical thickness	– 0.58 mm	1.79 (1.44–2.23)	< 0.001
Cortical cross-sectional area	– 39.5 mm^2	1.52 (1.19–1.95)	0.001
Cortical vBMD	– 66 mg HA/cm^3	1.71 (1.38–2.11)	< 0.001
Cortical bone mineral content	– 183 mg HA	1.91 (1.51–2.42)	0.001

SD, standard deviation; aBMD, areal bone mineral density; vBMD, volumetric BMD; HA, hydroxyapatite; FRAX, Fracture Risk Assessment Tool for calculation of the 10-year probability of a major osteoporotic fracture; Garvan, Fracture Risk estimate of the 10-year fracture risk for any fragility fracture. Both FRAX and Garvan estimates are log-transformed and included FN aBMD.

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Cortical porosity remained independently associated with fracture after adjustment for FN aBMD, FRAX, or Garvan estimates (1.39 [1.10–1.74], 1.53 [1.22–1.90] and 1.45 [1.16–1.81]) (Table 3). Cortical thickness remained independently associated with fracture after adjustment for FN aBMD, FRAX, or Garvan (1.46 [1.15–1.85], 1.47 [1.17–1.83], and 1.38 [1.10–1.73], respectively). When both cortical porosity and thickness were included in the same models with FN aBMD, FRAX, or Garvan estimates, cortical porosity remained associated with fracture, but cortical thickness did not. However, cortical cross-sectional area did not remain associated with fracture after adjustment for FN aBMD, FRAX, or Garvan estimates.

Discrimination of fracture. AUC for age and FN aBMD was 0.683, and AUC for FRAX alone was 0.679. Adding cortical porosity to FRAX improved the discrimination of fracture cases from controls over FRAX alone, and resulted in a slightly higher AUC of 0.705 (p = 0.051). Additionally, adding both cortical porosity and thickness to FRAX resulted in AUC of 0.709 (p = 0.031; Fig 1). For Garvan estimate alone AUC was 0.700, and adding both cortical porosity and thickness to the Garvan estimate resulted in a marginally higher AUC of 0.721 (p = 0.064).

Identification of fracture cases using Garvan, FRAX, and cortical bone parameters. FRAX estimate (>20%) identified 25.1% of women with fracture, Garvan estimate (>25%) identified 34.6%, cortical porosity (>80th percentile) identified 28.9%, and cortical thickness (<20th percentile) identified 27.5% (Table 4 and Fig 2). Sensitivity at these thresholds for

Table 3. Odds ratio (OR) and 95% confidence interval (CI) for nonvertebral fracture per standard deviation (SD) difference in each of cortical porosity, thickness, and cross-sectional area (CSA).

		Covariates in each of the models	OR (95% CI)
Cortical porosity	+ 4.01%	Age, height, weight, FN aBMD	1.39 (1.10–1.74)
		FRAX alone	1.53 (1.22–1.90)
		Garvan alone	1.45 (1.16–1.81)
Cortical thickness	– 0.58 mm	Age, height, weight, FN aBMD	1.46 (1.15–1.85)
		FRAX alone	1.47 (1.17–1.83)
		Garvan alone	1.38 (1.10–1.73)
Cortical CSA	– 39.5 mm^2	Age, height, weight, FN aBMD	1.06 (0.80–1.41)
		FRAX alone	1.02 (0.83–1.26)
		Garvan alone	0.94 (0.75–1.16)

FN aBMD; femoral neck areal bone mineral density; FRAX, Fracture Risk Assessment Tool for calculation of the 10-year probability of a major osteoporotic fracture; Garvan, Fracture Risk estimate of the 10-year fracture risk for any fragility fracture.
FRAX and Garvan estimates are used log-transformed, and both estimates included FN aBMD.

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FRAX and Garvan estimates and cortical porosity and thickness was 25%, 35%, 29%, and 28%, respectively, and specificity was 94%, 92%, 88%, and 88%, respectively. Combining FRAX with cortical porosity and thickness identified 45.5% and 42.7% of fracture cases, respectively, and combining Garvan with cortical porosity and thickness identified 51.2% and 48.3%, respectively. Measuring cortical porosity and thickness identified additional fracture cases than using FRAX alone (20.4% and 17.5%, respectively). Additionally, measuring cortical porosity and thickness also identified additional fracture cases than using Garvan alone (16.6% and 13.7%, respectively).

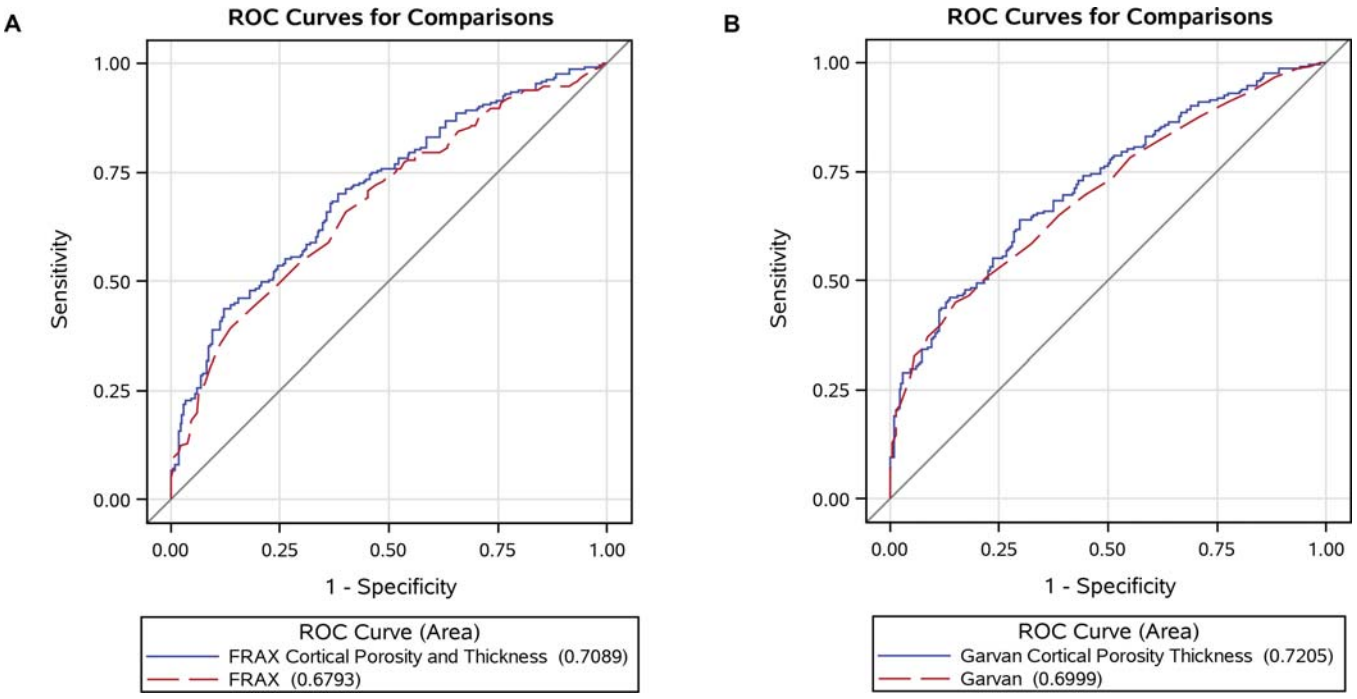

Fig 1. Area under the receiver operating characteristic curve (AUC) for comparison of (A) Fracture Risk Assessment Tool (FRAX) estimate before and after adding cortical porosity and thickness and (B) Garvan estimate before and after adding cortical porosity and thickness.

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Improved reclassification of fracture. After adding cortical porosity to FRAX, 43 women with fracture (20.4%) were correctly reclassified upward, 23 women without fracture (9.9%) were incorrectly reclassified upward, and NRI was 0.10 (95% CI: 0.03–0.18; $p = 0.005$) (Table 5). After adding cortical thickness to FRAX, 37 women with fracture (17.5%) were correctly reclassified upward, 25 women without fracture (10.8%) were incorrectly reclassified upward, and NRI was 0.07 (95% CI: 0.00–0.14; $p = 0.060$). After adding cortical porosity to Garvan, 35 women with fracture (16.6%) were correctly reclassified upward, 26 women without fracture (11.2%) were incorrectly reclassified upward, and NRI was 0.05 (95% CI: -02, 0.12; $p = 0.131$). After adding cortical thickness to Garvan, 29 women with fracture (13.7%) were correctly reclassified upward, 26 women without fracture (11.2%) were incorrectly reclassified upward, and NRI was 0.03 (95% CI: -0.04, 0.09; $p = 0.451$).

Discussion

We reported that women with fracture had higher cortical porosity, thinner cortices, and smaller cortical area. Cortical porosity and thickness remained associated with prevalent fracture, independent of FRAX and Garvan estimates, and increased the AUC. Measurement of cortical porosity and thickness identified additional women with fracture than those identified using FRAX and Garvan alone. Moreover, cortical porosity improved net reclassification of women with fracture compared with FRAX alone.

The development of FRAX and Garvan tools have improved the fracture risk prediction compared to the use of aBMD alone, and both tools are well validated [6,14,27–29]. However, both tools have limitations–specifically, the omission of other risk factors that are not included in FRAX and Garvan that can influence fracture risk and potentially enhance the predictive

Table 4. Sensitivity and specificity for each factor and for combinations with 95% confidence interval (CI).

	Sensitivity (%)	95% CI	Specificity (%)	95% CI
For each factor				
FRAX estimate >15%	45.0	38.2–52.0	80.2	74.3–85.0
FRAX estimate >20%	25.1	19.5–31.6	93.5	89.3–96.2
FRAX estimate >25%	12.3	8.35–17.7	97.8	94.8–99.2
Garvan estimate >15%	69.7	62.9–75.7	55.2	48.5–61.6
Garvan estimate >20%	46.5	39.6–53.4	81.9	76.2–86.5
Garvan estimate >25%	34.6	28.3–41.5	92.2	87.8–95.2
Cortical porosity >75th percentile (>45.1%)	34.1	27.8–41.0	83.2	77.6–87.6
Cortical porosity >80th percentile (>45.7%)	28.9	22.9–35.5	87.9	83.0–91.8
Cortical porosity >90th percentile (>48.2%)	16.1	11.6–21.9	95.3	91.4–97.5
Cortical thickness <10th percentile (<3.50 mm)	16.1	11.6–21.9	95.7	92.0–97.8
Cortical thickness <20th percentile (<3.75 mm)	27.5	21.7–34.1	87.5	82.4–91.3
Cortical thickness <25th percentile (<3.85 mm)	33.7	27.4–40.5	83.2	77.6–87.6
For combinations				
FRAX >20% or cortical porosity >80th percentile	45.5	38.7–52.5	83.6	78.1–88.0
FRAX >20% or cortical thickness <20th percentile	42.7	35.9–49.6	82.8	77.1–87.3
Garvan >25% or cortical porosity >80th percentile	51.2	44.3–58.1	81.0	75.3–85.7
Garvan >25% or cortical thickness <20th percentile	48.3	41.5–55.3	81.0	75.3–85.7
Cortical porosity >80th or thickness <20th percentile	39.8	33.2–46.8	79.7	73.9–84.6

FRAX: Fracture Risk Assessment Tool for calculation of the 10-year probability of major osteoporotic fracture with femoral neck areal bone mineral density (FN aBMD) included in the estimate. Garvan:10-year fracture risk estimate for any fragility fracture with FN aBMD included in the estimate.

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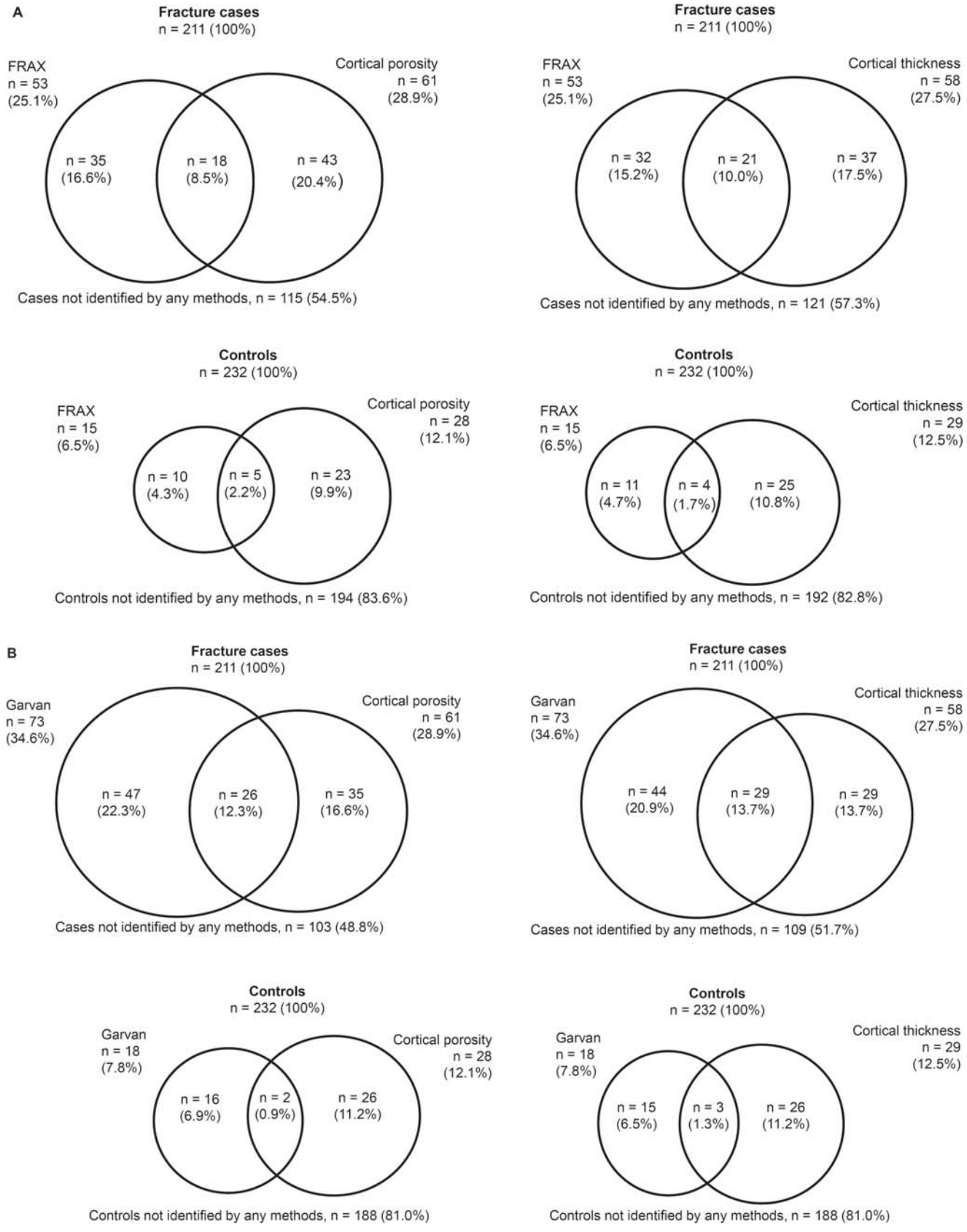
A
Fracture cases
n = 211 (100%)
FRAX
n = 53
(25.1%)
Cortical porosity
n = 61
(28.9%)
n = 35
(16.6%)
n = 18
(8.5%)
n = 43
(20.4%)
Cases not identified by any methods, n = 115 (54.5%)
Fracture cases
n = 211 (100%)
FRAX
n = 53
(25.1%)
Cortical thickness
n = 58
(27.5%)
n = 32
(15.2%)
n = 21
(10.0%)
n = 37
(17.5%)
Cases not identified by any methods, n = 121 (57.3%)
Controls
n = 232 (100%)
FRAX
n = 15
(6.5%)
Cortical porosity
n = 28
(12.1%)
n = 10
(4.3%)
n = 5
(2.2%)
n = 23
(9.9%)
Controls not identified by any methods, n = 194 (83.6%)
Controls
n = 232 (100%)
FRAX
n = 15
(6.5%)
Cortical thickness
n = 29
(12.5%)
n = 11
(4.7%)
n = 4
(1.7%)
n = 25
(10.8%)
Controls not identified by any methods, n = 192 (82.8%)
B
Fracture cases
n = 211 (100%)
Garvan
n = 73
(34.6%)
Cortical porosity
n = 61
(28.9%)
n = 47
(22.3%)
n = 26
(12.3%)
n = 35
(16.6%)
Cases not identified by any methods, n = 103 (48.8%)
Fracture cases
n = 211 (100%)
Garvan
n = 73
(34.6%)
Cortical thickness
n = 58
(27.5%)
n = 44
(20.9%)
n = 29
(13.7%)
n = 29
(13.7%)
Cases not identified by any methods, n = 109 (51.7%)
Controls
n = 232 (100%)
Garvan
n = 18
(7.8%)
Cortical porosity
n = 28
(12.1%)
n = 16
(6.9%)
n = 2
(0.9%)
n = 26
(11.2%)
Controls not identified by any methods, n = 188 (81.0%)
Controls
n = 232 (100%)
Garvan
n = 18
(7.8%)
Cortical thickness
n = 29
(12.5%)
n = 15
(6.5%)
n = 3
(1.3%)
n = 26
(11.2%)
Controls not identified by any methods, n = 188 (81.0%)

Fig 2. Venn diagrams show the number and proportion of woman identified using threshold for (A) Fracture Risk Assessment Tool (FRAX) estimate >20%, cortical porosity >80th percentile, and cortical thickness <20th percentile, and (B) Garvan estimate >25%, cortical porosity >80th percentile, and cortical thickness <20th percentile.

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ability of these tools. For example, TBS, a measurement derived from lumbar spine DXA images, is a well-documented risk factor for fracture, independent of aBMD and FRAX, in many cross-sectional and prospective studies, and can be included in the FRAX estimate [9,10]. However, the independent contribution from TBS to fracture risk is small [10].

To our knowledge, there is only one prospective study evaluating the predictive role of cortical porosity on incident fracture [13]. Cortical area and mass, but not porosity, at the distal tibia predicted any type of incident fracture in older men, assessed using HR-pQCT [13]. The lack of association of cortical porosity with incident fracture [28] was in contrast to previous studies suggesting cortical porosity was associated with prevalent fracture [12,14–16]. As most of the cases had wrist fractures, we showed that cortical porosity of the proximal femur was associated with prevalent wrist fractures. Another recent study showed that cortical porosity of the distal tibia was associated with prevalent hip fracture [16], but these studies did not investigate how cortical porosity is associated with vertebral fracture. As bone fragility is a general condition, we assume that cortical porosity, at any site, may be associated with any type of fracture.

Our group previously reported that sensitivity for fracture improved when cortical porosity was combined with FRAX, but over 50% of fracture cases were still unidentified from either of those measures [14]. In this study, we further explored whether inclusion of additional cortical parameters, such as cortical thickness and area, could provide additional information about fracture risk beyond the existing tools. Both cortical porosity and thickness were associated with fracture risk independent of aBMD, FRAX, and Garvan, and slightly increased the AUC. The sensitivity also increased and specificity remained high. However, when combing cortical porosity and thickness in the same model with FRAX and Garvan independently, cortical thickness was no longer associated with fracture, independent of cortical porosity. Moreover, about half of the fracture cases remained unidentified when these cortical parameters were added to Garvan or FRAX estimates. These results suggest that cortical porosity may be the most important cortical parameter and a potential predictor of fracture risk. The contribution from cortical thickness or cortical area to fracture risk seems to be modest. Further prospective studies are needed to determine whether cortical parameters provide independent information regarding fracture risk beyond FRAX and Garvan tools. Assessment of cortical porosity may be particularly of interest to identify the fracture risk in individuals without osteoporosis [14], and in those without a high Garvan or FRAX estimate.

Table 5. Reclassification of women with fracture in new models after adding cortical porosity or thickness to each of the original models including FRAX or Garvan alone.

	Net reclassification improvement (NRI)				
	Event	**Nonevent**	**Overall**	**95% CI**	**p-value**
FRAX + cortical porosity[a]	0.204	-0.099	0.10	0.03, 0.18	0.005
FRAX + cortical thickness[a]	0.175	-0.108	0.07	0.00, 0.14	0.060
Garvan + cortical porosity[b]	0.166	-0.112	0.05	-0.02, 0.12	0.131
Garvan + cortical thickness[b]	0.137	-0.112	0.03	-0.04, 0.09	0.451

FRAX: Fracture Risk Assessment Tool for calculation of the 10-year probability of major fracture. Garvan: Fracture Risk estimate of the 10-year fracture risk for any fragility fracture.

[a]Compared with FRAX alone.

[b]Compared with Garvan alone.

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In order to improve the sensitivity and still achieve high specificity, we explored the tradeoff for FRAX and Garvan at selected thresholds above 15%, 20%, and 25%, respectively. For FRAX, we considered a threshold >20% as the best cutoff; although the sensitivity was 25%, the specificity was 94%. When using a Garvan threshold >20%, the sensitivity was 47% (which agreed with a previous report [28]) and specificity was 82%. However, we wanted a threshold with better specificity (at least 85–90%) for each of the traits considered for further analysis in order to minimize the number of false positives. Using a Garvan threshold >25% was therefore considered as an optimal cutoff in the current data, and although the sensitivity was 35% the specificity was 92%. Combinations of risk factors increased sensitivity and maintained high specificity; however, some specificity was lost.

Prior fractures are included in both the FRAX and Garvan tools. However, while Garvan includes the number of prior fractures, FRAX does not. This may capture additional risk and contribute to differences in the performance between these tools. Another possible explanation of differences could be that the fall history is included in Garvan, but not in FRAX [7,10,28]. However, only about 5% of falls in the elderly result in a fracture [30,31]. In this study, those with one or more falls had no higher risk for fracture than those without. Secondary osteoporosis due to chronic diseases or early menopause are well-known risk factors for fracture and are included in FRAX [5,6]. However, the individual FRAX estimate remained unchanged after inclusion of secondary osteoporosis because the risk of fracture related to secondary osteoporosis is captured by aBMD [5]. Although FRAX and Garvan tools were designed to predict incident fracture prospectively, we believe it is useful to evaluate associations in retrospective settings, as it may provide interesting suggestions on risk factors that could be important to study in future prospective studies [12,32]. Ideally, we should have included vertebral fractures and more hip fractures. However, most of those with hip fracture had metal in the hip region and could not be included as metal makes noise in the CT images at both sides. Additionally, most of the patients suffering a vertebral fracture were not admitted to the hospital for an x-ray verification of fracture. The inclusion of largely wrist and humerus fractures are still of interest because these are typical osteoporotic fractures [12,29].

The strength of this nested case-control study was that it was based on a general population, fractures were x-ray verified, and cortical parameters were quantified at the proximal femur, a central site, and a common site for the most serious fragility fracture. The benefit and novelty of using the non-threshold-based software was how it was different from traditional porosity measurements. Porosity was presented here as a void fraction, and not a visually quantifiable estimate based on size and dimension. Our measure was more inclusive by encompassing porosity of both the compact cortex and TZ, and by taking into account the partial volume effect. As a result, the values of porosity were higher [12,14,18,19,24,25,33] than previously reports using other methods [13,15–16]. Studies using traditional methods to quantify porosity presented ranges from 1% to 15% likely due to only quantifying porosity of the compact cortex and porosity of completely empty voxels [13,15–16]; thus, this threshold-based image analysis underestimates porosity [24,33].

This study had several limitations. The retrospective case-control design may have introduced selection bias. The index fracture occurred at a median of 6.6 y before the women had their measurements performed. In addition, most of the women with hip fracture could not be included as metal can generate noise in the CT images. Lastly, the subtrochanteric region contained little trabecular bone, so its contribution to fracture risk could not be evaluated in this data.

In conclusion, cortical porosity and thickness were associated with increased odds for fracture, independent of aBMD, FRAX, and Garvan estimates, and slightly improved the AUC. Adding cortical porosity to existing tools may be helpful to improve fracture risk assessment

beyond existing FRAX and Garvan tools, and help to identify those patients who will benefit from treatment. Further prospective studies are needed to determine whether cortical porosity or other bone traits predict fracture. Moreover, scanning procedures with low radiation, low cost, and low demand on the facilities offering these measurements need to be developed.

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Author Contributions

Conceptualization: Rita Kral, Marit Osima, Tove T. Borgen, Roald Vestgaard, Elin Richardsen, Åshild Bjørnerem.

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Formal analysis: Åshild Bjørnerem.

Funding acquisition: Rita Kral, Elin Richardsen, Åshild Bjørnerem.

Methodology: Rita Kral, Elin Richardsen, Åshild Bjørnerem.

Project administration: Åshild Bjørnerem.

Visualization: Rita Kral.

Writing – original draft: Rita Kral, Åshild Bjørnerem.

Writing – review & editing: Rita Kral, Marit Osima, Tove T. Borgen, Roald Vestgaard, Elin Richardsen, Åshild Bjørnerem.

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Paper II

Women with Fracture, Unidentified by FRAX, but Identified by Cortical Porosity, have a Different Patient Profile that Contribute to Fracture Risk

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Disclosures

The authors have nothing to disclose.

ABSTRACT

The Fracture Risk Assessment Tool (FRAX) is widely used to identify individuals at increased risk for fracture. However, cortical porosity is associated with risk for fracture independent of FRAX and is reported to improve the net reclassification of fracture cases. 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 score 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 fracture-free controls in Tromsø, Norway, using StrAx1.0 software. Of 211 fracture cases, FRAX score >20% identified 53 women (sensitivity 25.1% and specificity 93.5%), while cortical porosity cut-off >80th percentile identified 61 women (sensitivity 28.9% and specificity 87.9%). The 43 (20.4%) additional fracture cases identified by high cortical porosity alone, had lower FRAX score (12.3 vs. 26.2%) than those identified by FRAX alone, they were younger, had higher FN aBMD (806 vs. 738 mg/cm^2), and fewer had a prior fracture (23.3 vs. 62.9%), all $p < 0.05$. They had higher cortical porosity (48.7 vs. 42.1%), thinner cortices (3.75 vs. 4.12 mm), larger total and medullary cross-sectional areas (669 vs. 593 and 245 vs. 190 mm^2), higher cross-sectional moment of inertia (2619 vs. 2388 cm^4) and lower cortical and total volumetric BMD (942 vs. 1053 and 586 vs. 699 mg HA/cm^3), all $p < 0.001$. Fracture cases, unidentified by FRAX, but identified by cortical porosity, had an architecture where the positive impact of larger bone size did not offset the negative effect of thinner cortices with increased porosity. A measurement of cortical porosity may be a marker of a patient profile that captures additional fracture risk components, not captured by FRAX.

Key words: bone size, cortical porosity, fracture, FRAX, postmenopausal women

Introduction

The Fracture Risk Assessment Tool (FRAX) is widely used in many countries and has improved fracture risk prediction compared to areal bone mineral density (BMD) alone (1-3). Despite of the inclusion of several well-known risk factors for fracture, this tool has limitations in terms of lack of sensitivity (4, 5). For this reason, there are ongoing discussions concerning which of the included risk factors may not be needed, as well as which factors could be added to FRAX to improve the fracture prediction (3). Many bone features contribute to bone strength, such as the bone architecture and geometry (6, 7). A larger size is important for bone strength, because the resistance to bending increases to the fourth power of its radius (8). Moreover, deterioration of both the cortical as well as the trabecular architecture compromises bone strength (8, 9). However, in an experimental study, which examined the contribution of cortical versus trabecular bone using biomechanical testing, trabecular bone contributed to only 7% of bone strength in the femoral neck (10). Trabecular bone score can be used in the FRAX calculation, but it results in only a modest improvement of fracture risk prediction (3, 11). Cortical porosity is a potential risk factor for fracture as cortical bone constitute 80% of the skeleton (12), and contribute over 90% to bone strength (10), still, cortical porosity or other cortical bone parameters are not included in the FRAX.

Several cross-sectional studies have reported that increased cortical porosity assessed using high-resolution peripheral quantitative computed tomography (HR-pQCT) and clinical CT technology, is associated with prevalent fracture in women and men (13-17). In contrast, no association was confirmed between cortical porosity at distal tibia and fracture risk in a prospective study of elderly men using HR-pQCT software (18). In another study using HR-pQCT and Strax1.0 software, cortical porosity of the inner transitional zone at ultra-distal radius was associated with incident fracture in postmenopausal women independent of femoral neck (FN) aBMD and FRAX score, but only marginally after adjustment for ultra-distal radius

aBMD (19). Our research group has previously reported that increased cortical porosity at the proximal femur was associated with fracture independent of FN aBMD and FRAX (15, 20). Using a cortical porosity threshold >80th percentile identified 20% additional fracture cases who were unidentified by FRAX, and improved the net reclassification of fracture cases (20). This suggests that a measurement of cortical porosity captures other important skeletal properties not captured by the FRAX score. The reasons why some women are identified by FRAX, while others are identified by a measurement of cortical porosity is not clear. To the best of our knowledge, no previous study have reported the patient profiles of the additional individuals with fractures who are identified by cortical porosity independently 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.

Materials and methods

Study population

The Tromsø Study is a single-center, population-based study in Northern Norway, which conducted six surveys between 1974 and 2008 (21). During the Tromsø 4 survey in 1994–95, 37,558 eligible inhabitants in Tromsø over 24 years old were invited to participate, and 27,158 (72%) agreed. Within these participants, all nonvertebral fractures that occurred between January 1, 1994 and January 1, 2010 were registered from the University Hospital of North Norway, Tromsø x-ray archives (22). 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 and identified 1250 women from the x-ray-based fracture registry that suffered at least one fracture of the hip, wrist, or proximal humerus after the age of 50 years (15, 20, 23-25). We invited all 760 women who were still alive and living in Tromsø. All women who were willing to participate had a pre-screening

phone call to determine whether they were eligible for participation in accordance with the inclusion and exclusion criteria. Those who were premenopausal, received bisphosphonates, or had hip prostheses or metal screws in the hip region were excluded from the study. Since metal on one hip can create noise in the CT images on both sides, many women with a hip fracture could not be included unless they had the metal removed. After screening, 264 fracture cases were included in the study (15, 20, 23, 24). Age-matched, fracture-free women, who were within the same 5-year age groups, were randomly selected from the Tromsø 4 participants, and 1186 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 due to motion artifacts during CT scanning. This left 443 women included in the final analyses: 232 controls and 211 fracture cases (4 hip, 181 wrist, and 26 proximal humerus). The median time since their last fracture was 6.6 years (range, 1–25). All variables included in the analysis were based on information obtained at the time of study enrollment between November 2011 and January 2013. All participants provided written informed consent. The study was approved by the Regional Committee for Medical and Health Research Ethics (REK Sør-Øst, 2010/2282) and was conducted in accordance with the World Medical Association Declaration of Helsinki.

Variables and measurements

At enrollment of the study, the participants filled in a questionnaire that included information concerning all fractures after the age of 50 years (number and type of fracture), diseases, use of medication and lifestyle. Height and weight were measured while wearing light clothing and without shoes. Body mass index (BMI) was calculated as weight/height^2. FN aBMD was measured using dual-energy x-ray absorptiometry (DXA) (GE Lunar Prodigy, Lunar Corporation, Madison, WI, USA) and the coefficients of variation (CV) was 1.7%.

We entered the 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/). An age of 90 years was entered into the calculation tool in women older than 90 years of age, and we included FN aBMD in the calculation of FRAX score (20). The index fractures used as inclusion criteria for this study were not included as a "previous" fracture in the calculation of the FRAX score, because the aim was to assess the 10-year probability of fracture before the event, not the probability of fracture after this event (14, 15). Whereas the "previous fractures" (before the index fracture) and "subsequent fractures" (after the index fracture) were used equally in the calculation of FRAX score (20).

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 (15). 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 (15). 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 (26). Quality control was carried out by scanning a phantom containing rods of hydroxyapatite (QRM Quality Assurance in Radiology and Medicine GmbH, Moehrendorf, Germany). The CT images were sent to Melbourne, Australia, and analyzed by collaborators, who were blinded to the fracture status, using the StrAx1.0 software (StraxCorp Pty Ltd, Melbourne, Australia). 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 as shown in Fig. 1 (15, 27).

The StrAx1.0 software is a non-thresholding method that automatically selects attenuation profile curves and segments the bone within the ROI into its compartments, the compact-appearing cortex, outer (OTZ) and inner transitional zone (ITZ), and trabecular

compartment (28). This was achieved by quantification of the attenuation produced by background (i.e., muscle) and fully mineralized bone matrix, which has a density of 1200 mg hydroxyapatite (HA)/cm^3) and assigned a value of 100% (27, 28). Voxels that were completely empty and had an attenuation equivalent to background were assigned a value of 0%. The volume fraction of a voxel that is void (i.e., porosity) is 100% minus the mineralized bone matrix fraction. Once deposited, osteoid is rapidly mineralized to become 'bone', reaching 80% of full mineralization (1200 mg HA/cm^3) within a few days. Voxels with attenuation values of 80% are unlikely to contain a pore or part of a pore, because porosity results in voxel attenuation values < 80% of the maximum. Variations in attenuation within 80% to 100% of full mineralization are likely to reflect heterogeneity in secondary mineralization of the matrix, thus these voxels are excluded from the calculation of porosity (28). Voxels with attenuation < 80% may contain a pore or part of a pore (28).

Porosity within the total cortex and each cortical compartment was quantified automatically throughout the ROI using the StrAx1.0 software (15). The porosity quantified by this algorithm is the proportion of emptiness within each voxel or the fraction of the bone that is void, with CV of 0.3-2.3% (15). StrAx1.0 quantifies porosity in low-resolution images (15, 27), as in high-resolution images (13, 28, 29), even though pores are not visible. It is a density-based, indirect measure of porosity, and the size and number of pores are not determined (15, 28, 30). Of the total cortex at this subtrochanteric site, 70.0% was compact-appearing cortex, while 22.3% and 11.7% was OTZ and ITZ, respectively. The agreement (R^2) between CT and HR-pQCT ranged from 0.86 to 0.96 for quantification of porosity (ranging from 40 to 95%), at the same femoral subtrochanteric site (15, 27). The StrAx1.0 software quantifies porosity as a fraction of void, regardless of size of the pores, and indirectly captures porosity produced by large and small pores. It is more inclusive than traditional methods by capturing porosity of the compact cortex and the TZ, and by taking into account the partial volume effect by including

void within completely empty and partly empty voxels, and the porosity is therefore higher than what is reported using other methods (27, 28, 30).

Statistical analyses

We present mean and standard error of the mean (SE) in four groups. Group 1: 35 fracture cases identified by high FRAX score (threshold >20%), but unidentified by high cortical porosity (threshold >80th percentile). Group 2: 43 fracture cases unidentified by high FRAX score, but identified by high cortical porosity. Group 3: 115 fracture cases unidentified by both high FRAX score and cortical porosity. Group 4: 232 age-matched fracture-free controls. The characteristics the women within each of the groups were compared using age-adjusted analysis of variance, and the bone parameters were compared after additionally adjustment for height and weight. We used SAS Software, v9.4 (SAS Institute Inc., Cary, NC, USA) and $p < 0.05$ was considered significant.

Results

Of all 211 fracture cases, FRAX score >20% identified 53 women, with a sensitivity of 25.1% and specificity of 93.5%, while a measurement of cortical porosity with cut-off >80th percentile identified 61 women, with a sensitivity of 28.9% and specificity of 87.9% (Fig. 2). Of 211 fracture cases, 35 (16.6%) (Group 1) were identified only by high FRAX score, and 43 (20.4%) (Group 2) were identified only by high cortical porosity. There was an overlap for 18 (8.5%) women with fracture who had both high FRAX score and high cortical porosity, and 115 (54.5%) (Group 3) fracture cases were unidentified by either.

Characteristics of fracture cases identified by high FRAX score alone

Fracture cases identified by high FRAX score alone, had a higher FRAX score (26.2 vs. 12.3%), were 4 years older (71.7 vs. 67.6), had 8.4% lower FN aBMD (738 vs. 806 mg/cm^2), and more had a prior fracture (22 vs. 10%) and a parental history of hip fracture (16 vs. 4%) compared to those identified by high cortical porosity alone (all $p < 0.05$, Table 1 and Fig. 3). Otherwise, the FRAX score and the risk factors included in FRAX differed little between Group 2, 3 and 4, except for the higher FN aBMD in controls (Group 4) than in all other groups, $p < 0.001$.

Characteristics of fracture cases identified by high cortical porosity alone

Women with fracture who were identified by high cortical porosity alone, had 15.7% higher porosity of the total cortex (48.7 vs. 42.1%), 14.5% higher porosity of the compact cortex (38.7 vs. 33.8%) and 6.7% higher porosity of the OTZ (47.5 vs. 44.5%), all $p < 0.001$, but not higher porosity of the ITZ (83.9 vs. 84.3%) than those identified by high FRAX score alone (Table 1, Fig. 4). They had 9.0% thinner cortices (3.75 vs. 4.12 mm), 28.9% larger medullary cross-sectional area (CSA) (245 vs.190 mm^2), 12.8% larger total CSA (669 vs. 593 mm^2), and 9.7% higher cross-sectional moment of inertia (CSMI) (2619 vs. 2388 cm^4), 10.5% lower cortical volumetric BMD (vBMD) (942 vs. 1053 mg HA/cm^3), and 16.2% lower total vBMD (586 vs. 699 mg HA/cm^3), all $p < 0.001$. Otherwise, bone traits differed little between Group 1, 3 and 4.

Discussion

We report that fracture cases unidentified by FRAX but identified by cortical porosity, had a different patient profile than the fracture cases identified by FRAX. Those who were identified by cortical porosity alone, had lower FRAX score, were younger, with higher FN aBMD, fewer had a prior fracture and parental history of hip fracture, and they had a relatively larger bone size, larger medullary cavity, thinner and more porous cortices at the femoral subtrochanteric site, than fracture cases identified by FRAX alone. From these results we infer that a

measurement of cortical porosity capture additional fracture risk components, that is not captured by FRAX.

As expected, fracture cases identified by FRAX were older, with lower FN aBMD, and more had a prior fracture, as these are the key components of the FRAX tool. We further confirmed that FRAX captured the risk factors related to diseases as rheumatoid arthritis and oral use of corticosteroids. Still, only 25% of the fracture cases were identified by FRAX, and several other bone traits reflecting risk components of the multifactorial condition bone fragility seem not to be well captured by this tool (5). A proportion of only 8.5% of the fracture cases were identified by both FRAX and cortical porosity in this study. This small overlap suggests that there probably are major differences between the characteristics of these two groups of fracture cases. In addition, cortical porosity improved the net reclassification of fracture cases when cortical porosity was added to FRAX, which support the notion that cortical porosity makes an important and independent contribution to identification of fracture risk (20).

Of the 75% of fracture cases who were unidentified by FRAX, 20% were identified by cortical porosity. They did not have the characteristic risk factors identified by FRAX, but they had a set of bone parameters that differed from those identified by FRAX. In addition to high cortical porosity, they had thinner cortices, both are well-known risk factors for fracture (31). They had a larger total bone CSA and increased CSMI, which would be expected to reduce the risk for fracture (8, 9). The increased risk for fracture in these women, suggest that the strength gained by larger bone size, did not offset the strength lost by the thinner cortices with higher cortical porosity (24). Larger bone size is associated with higher cortical porosity (13, 32) and taller individuals who on average have longer and wider bones, have increased risk for fracture (33, 34). The increased porosity combined with relatively thinner cortices, may partly explain why taller individuals, despite of their larger bone size, have increased risk for fracture (13, 32).

Fracture cases identified by high cortical porosity, had lower total bone vBMD, so their larger bones were more empty, because they had thinner cortices with higher porosity, smaller cortical CSA/total CSA, and thus larger medullary CSA/total CSA, than other fracture cases and controls. Our research group has reported that women with fracture had increased bone turnover markers, and the increased levels of bone turnover markers were associated with higher cortical porosity, thinner cortices, larger marrow cavity and larger bone size (24). Bone turnover occurs on all endosteal surfaces; intracortical, endocortical and trabecular surfaces (12). Increased bone turnover i) on the intracortical surfaces results in larger pores and increased porosity within the cortical compartment, ii) on the endocortical surfaces results in thinning of the cortex, and iii) on the trabecular surfaces it results in thinning and loss of trabeculae (35, 36). All these changes result in reduced bone strength (6, 12). A measurement of cortical porosity may be a marker for this whole set of the above-mentioned bone traits, and it can be useful for identification of individuals at risk for fracture, beyond those identified by FRAX.

Women with fracture identified by high cortical porosity, had higher porosity in both the compact-appearing cortex and the outer transitional zone compared to the other three groups. The increased porosity in the outer part of the cortex may cause a greater loss of strengths as it is located more distant to the neutral axis, given the high stress on the outer part of the cortex during a trauma (35). This may partly explain their increased risk for fracture. Cortical bone microstructure, especially cortical porosity has a major impact on bone strength (37, 38). An increase in porosity from 4 to 20% decrease the ability of bone to resist fracture by three-fold (39). In addition, 70-80% of the variation in stiffness as examined in the femoral cortex, can be explained by changes in cortical porosity (38, 40). High cortical porosity can appear as giant pores in cross-sectional images, which decrease the ability of the cortex to withstand stress (41) and resist crack propagation especially under tensile loading (42-44). Moreover, microcracks located near intracortical pores compromise fracture resistance (45).

Different genetic variants associated with cortical and trabecular bone traits are identified (46), and up to 80% of the variance in cortical and trabecular microarchitecture are determined by genetic factors (29). The implication of those findings is that the heterogeneous pathophysiology behind bone fragility, is not only a result of age-related changes, but genetic variation established during growth early in life, which may contribute to fracture risk in younger age (35). In addition, the fracture cases who unidentified by either high FRAX or cortical porosity, may have other risk factors for fracture beyond those we have quantified in this study, or their fracture might have occurred due to the trauma involved during their fall.

The strength of this nested case-control study is that it is based on a general population, x-ray verified fractures, and the bone parameters are quantified at the proximal femur, a central site. The benefit and novelty of using this non-threshold based software lie in how it is different from traditional porosity measurements. It is more inclusive than traditional methods by capturing porosity not only of the compact cortex but also the TZ, and by taking into account the partial volume effect (28). The study has several limitations. The index fracture occurred at a median of 6.6 years before the women had their measurements were performed, and most of the women with hip fractures could not be included, as metal can generate noise in the CT images. The subtrochanteric region contained little trabecular bone, so its contribution to fracture risk could not be evaluated, and StrAx1.0 software is vulnerable to motion artifact.

In conclusion, fracture cases identified by high cortical porosity alone had a different patient profile compared to those identified by the FRAX alone. In the relatively younger fracture cases unidentified by FRAX, the larger bone size did not offset the thinner cortices with higher cortical porosity. Such a patient profile is of interest for three reasons, firstly these women broke their bones without having the traditional risk factors as high age and low aBMD, secondly, they constitute a separate group of women that otherwise would not have been identified by calculation of FRAX, and thirdly, we have recently reported that cortical porosity

improved the net reclassification of fracture cases (20). This may explain why some women break their bone in relatively younger age, and may help identify those who are at risk for fracture before they have their first fracture. A measurement of cortical porosity may be a marker of a patient profile, which can identify additional women at risk for fracture, not captured by FRAX. Adding cortical porosity to FRAX may be of help to improve fracture risk assessment, not only for secondary, but also primary fracture prevention.

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The Tromsø Study provided access to data. Staff at the Department of Research at the University Hospital of North Norway (UNN) recruited women, obtained consent and questionnaires, collected blood samples and performed the DXA scanning. Staff at the Department of Radiology and Department of Radiation, UNN performed CT scanning of the patients, organized the radiation procedures and the CT images, and Strax Corp, Melbourne analyzed the CT images.

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Figure legends

Fig. 1. 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. Reproduced with permission from John Wiley and Sons, Zebaze et al. J Bone Miner Res. 31 (2016) 1827–1834 (27).

Fig. 2. Fractures cases identified by high cortical porosity alone, high Fracture Risk Assessment Tool (FRAX) score alone, the overlap, and cases who were unidentified by any measurements.

Fig. 3. Fracture Risk Assessment Tool (FRAX) score, age, femoral neck areal bone mineral density (FN aBMD) and proportion with a prior fracture in these four groups. Group 1: fracture cases identified by FRAX score >20% but unidentified by cortical porosity >80th percentile. Group 2: fracture cases unidentified by high FRAX score, but identified by high cortical porosity. Group 3: fracture cases unidentified by either. Group 4: controls.

Fig. 4. Cortical porosity, cortical thickness, total and medullary cross-sectional area (CSA) and Cross-sectional Moment of Inertia (CSMI) at the femoral subtrochanteric site in four groups. Group 1: fracture cases identified by FRAX score >20% but unidentified by cortical porosity >80th percentile. Group 2: fracture cases unidentified by high FRAX score, but identified by high cortical porosity. Group 3: fracture cases unidentified by either. Group 4: controls.

Table 1. Characteristics of the additional fractures cases identified by high FRAX score alone, those identified by high cortical porosity alone, cases who were unidentified by either, and the controls

	Group 1 Cases with FRAX score >20%	**Group 2 Cases with Porosity >80th percentile**	**Group 3 Cases, not identified by any method**	**Group 4 Fracture free Controls**
n	35	43	115	232
	Mean (SE)	Mean (SE)	Mean (SE)	Mean (SE)
FRAX score (%)	**26.2 (1.2)[c,f,i]**	12.3 (0.6)	11.2 (0.3)	10.8 (0.3)
Age (years)	**71.7 (1.3)[a,f,h]**	67.6 (1.1)	66.6 (0.7)	68.3 (0.4)
Height (cm)	162.3 (1.2)[g]	164.1 (0.8)[h]	162.8 (0.6)	161.2 (0.4)
Weight (kg)	69.7 (1.3)	70.3 (1.8)	69.3 (1.0)	70.0 (0.7)
Body mass index (BMI) (kg(m²)	26.6 (0.6)	26.1 (0.7)	26.1 (0.4)	27.0 (0.3)
Physical activity (hours/week)	2.2 (0.3)	2.8 (0.3)	2.7 (0.2)	2.5 (0.1)
Femoral neck aBMD (mg/cm²)	**738 (11.9)[b,f,i]**	806 (12.7)[i]	825 (9.1)[i]	860 (7.3)
History of previous fracture, n (%)	**22 (62.9)[c,f]**	10 (23.3)	18 (15.7)	0
Parental hip fracture history, n (%)	**16 (45.7)[c,f,i]**	4 (9.3)	12 (10.4)	37 (16.0)
Currently smoking, n (%)	6 (17.1)	7 (16.3)	14 (12.2)	24 (10.3)
Rheumatoid arthritis, n (%)	**5 (14.3)[e,h]**	2 (4.7)	3 (2.6)	8 (3.4)
Oral corticosteroid use, n (%)	**6 (17.1)[c,f,i]**	1 (2.3)	1 (0.9)	2 (0.9)
Diabetes mellitus type 2, n (%)	3 (8.6)	2 (4.7)	4 (3.5)	13 (5.6)
Self-reported good health, n (%)	20 (58.8)	34 (79.1)	79 (69.3)	165 (71.1)
Take calcium supplements, n (%)	8 (22.9)	11 (25.6)[d]	22 (19.1)	28 (12.1)
Take supplements Vitamin D n, (%)	30 (85.7)	32 (74.4)	86 (74.8)	166 (71.6)
Femoral subtrochanteric parameters				
Porosity total cortex (%)	42.1 (0.4)[c]	**48.7 (0.4)[f,i]**	41.4 (0.2)	41.7 (0.2)
Porosity compact cortex (%)	33.8 (0.3)[c]	**38.7 (0.3)[f,i]**	33.8 (0.2)	34.3 (0.2)
Porosity outer transitional zone (%)	44.5 (0.4)[c,g]	**47.5 (0.3)[f,i]**	44.8 (0.2)[g]	45.3 (0.1)
Porosity inner transitional zone (%)	84.3 (0.2)	83.9 (0.3)	84.0 (0.1)	84.2 (0.1)
Cortical thickness (mm)	4.12 (0.08)[c,g]	**3.75 (0.09)[f,i]**	4.27 (0.04)	4.36 (0.04)
Cortical vBMD (mg HA/cm³)	1053 (6.7)[c]	**942 (6.6)[f,i]**	1065 (3.8)	1059 (3.7)
Cortical CSA (mm²)	403 (6.4)[b,i]	424 (5.8)	410 (3.7)[g]	417 (2.6)
Cortical CSA/Total CSA	0.68 (0.01)[c,d,h]	**0.64 (0.01)[f,i]**	0.71 (0.005)	0.72 (0.003)
Trabecular BV/TV (%)	0.24 (0.03)	0.36 (0.04)[d]	0.24 (0.02)	0.27 (0.02)
Medullary CSA (mm²)	190 (7.2)[c,g]	**245 (10.5)[f,i]**	169 (4.3)	164 (2.8)
Total bone vBMD (mg HA/cm³)	699 (12.5)[c,d,g]	**586 (12.6)[f,i]**	743 (7.9)	750 (5.9)
Total bone CSA (mm²)	593 (10.0)[c]	**669 (12.2)[f,i]**	578 (5.9)	582 (4.0)
Cross-sectional Moment of Inertia	2388 (56)[c]	**2619 (56)[f,i]**	2332 (31)	2361 (21)

Values are mean (SE) or number (%). SE = standard error of the mean.

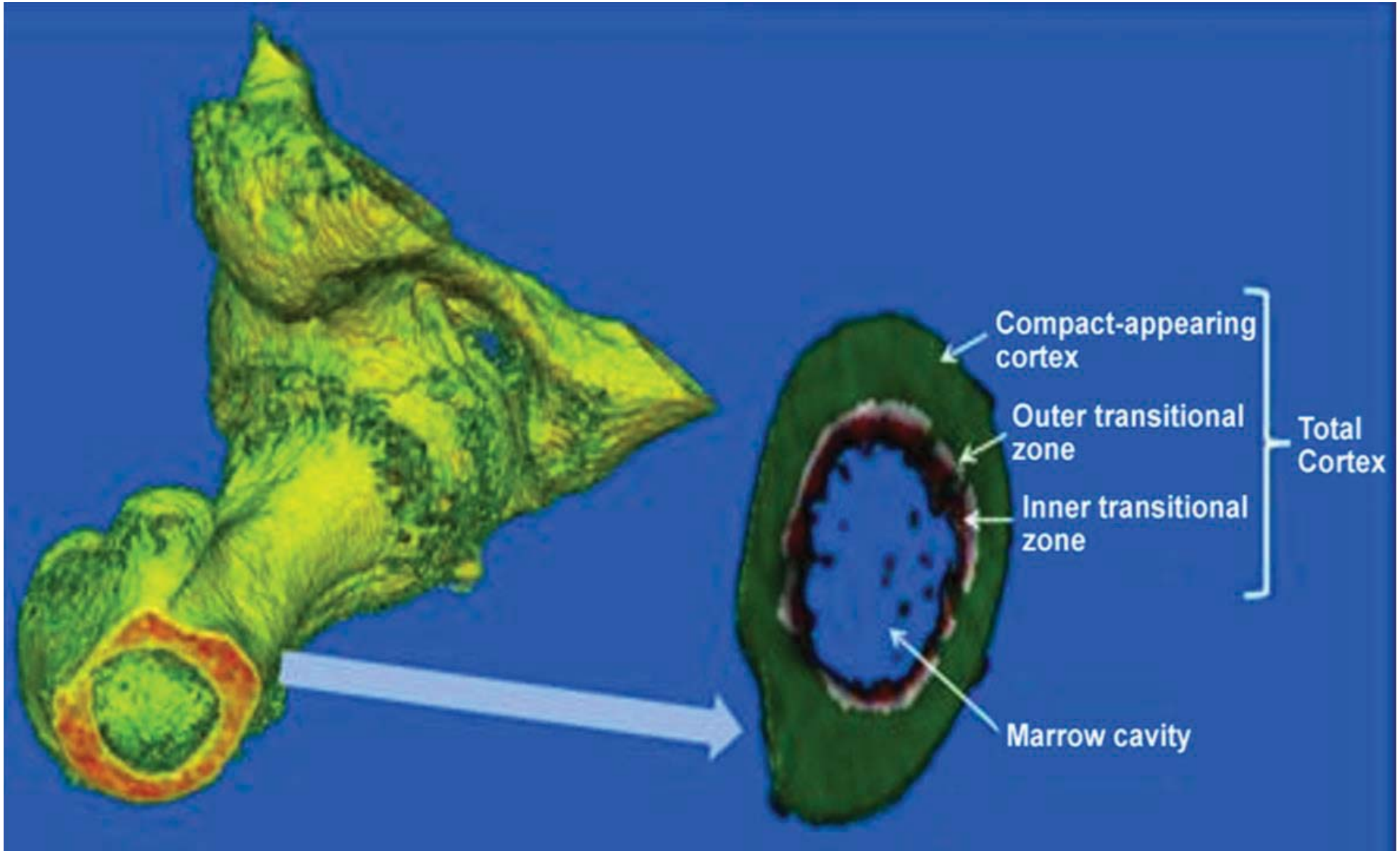
FRAX = Fracture Risk Assessment Tool for calculation of the 10-year probability of a major osteoporotic fracture; aBMD = areal bone mineral density; vBMD = volumetric bone mineral density; HA = hydroxyapatite; CSA = cross sectional area; BV/TV = bone volume per tissue volume.

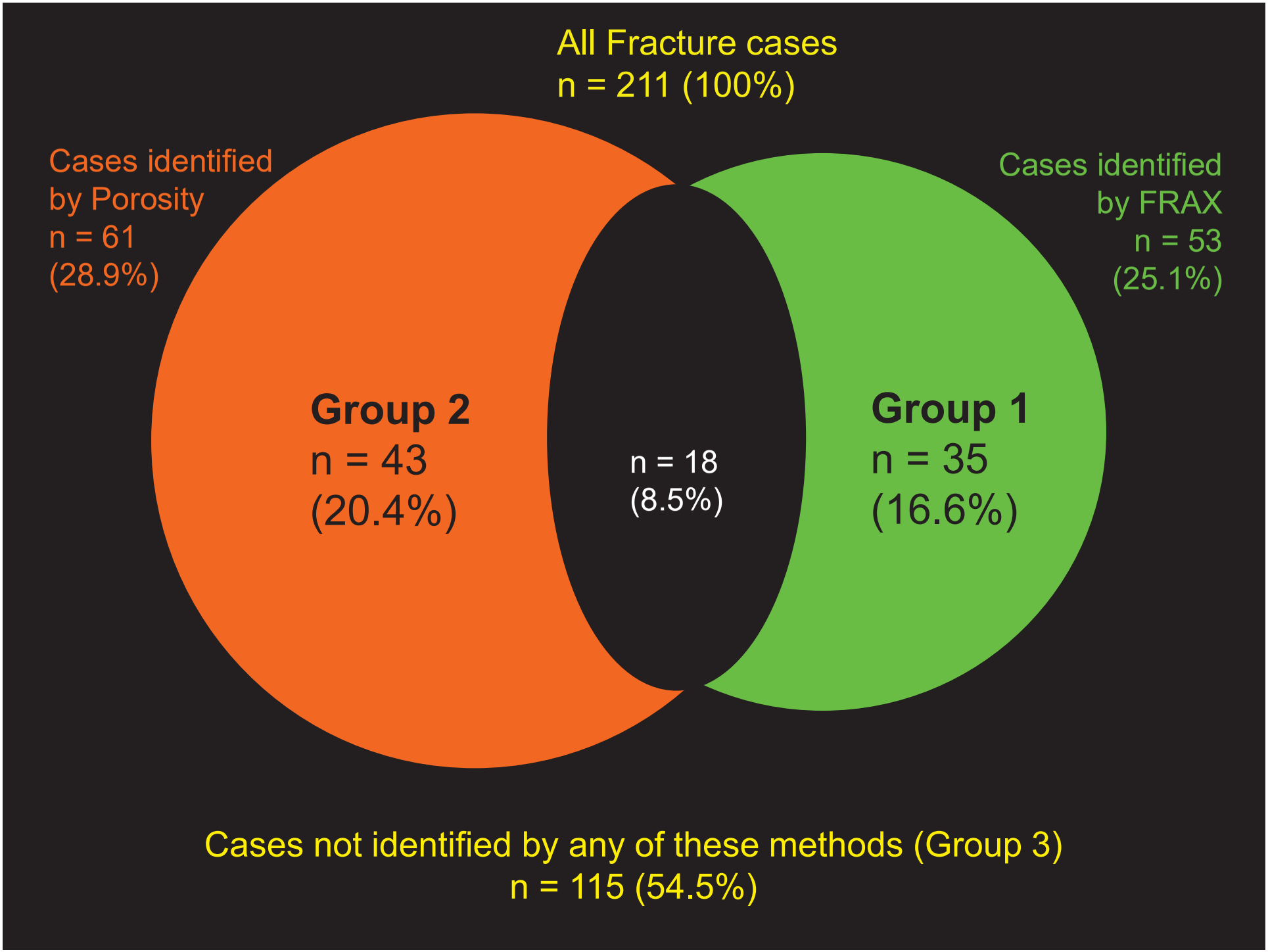
Analysis of variance was used for comparisons of the groups, all comparisons were adjusted for age, and comparisons of bone parameters were additionally adjusted for height and weight.

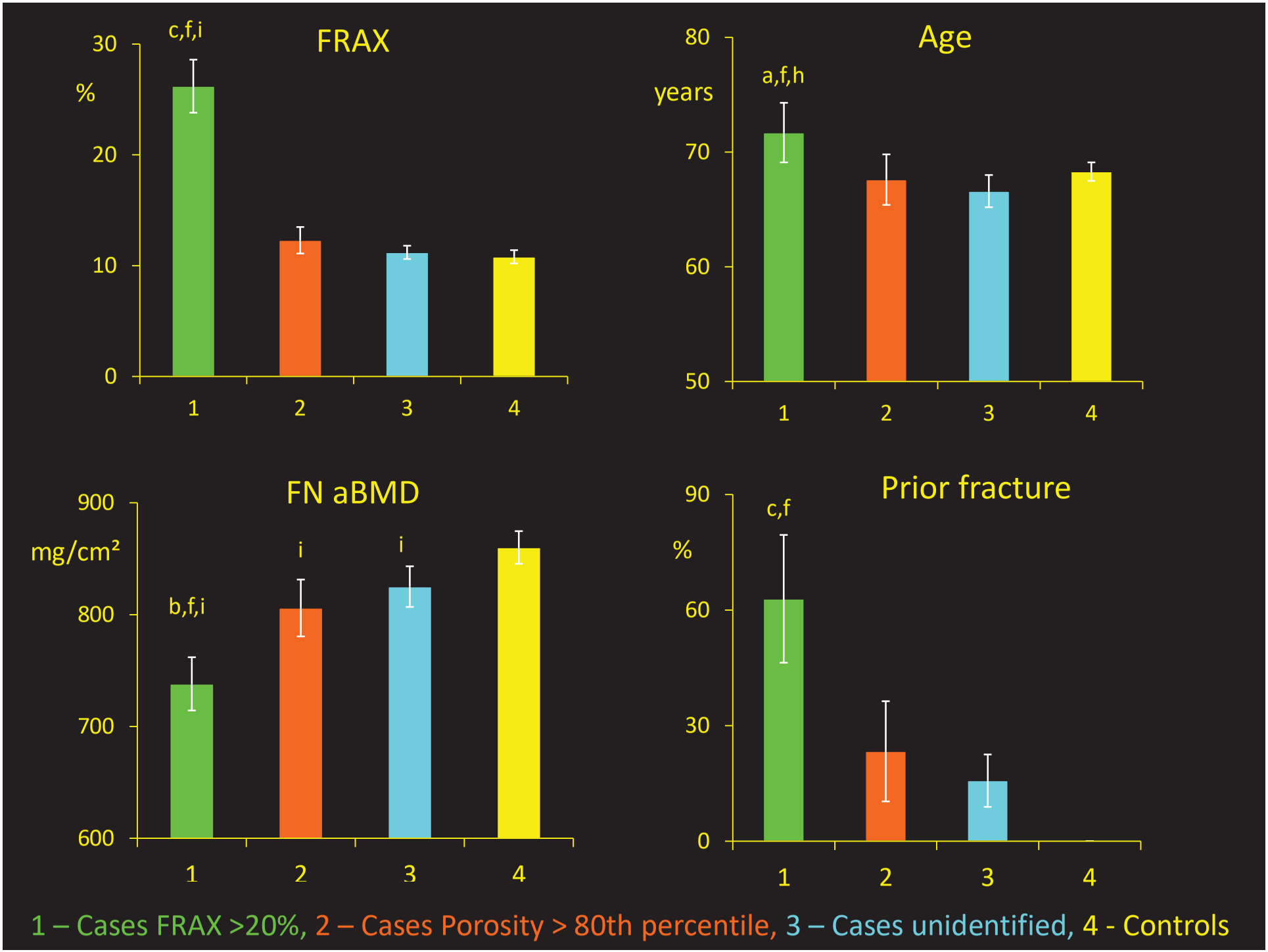
[a]$p < 0.05$, [b]$p < 0.01$, [c]$p < 0.001$ compared to group 2,

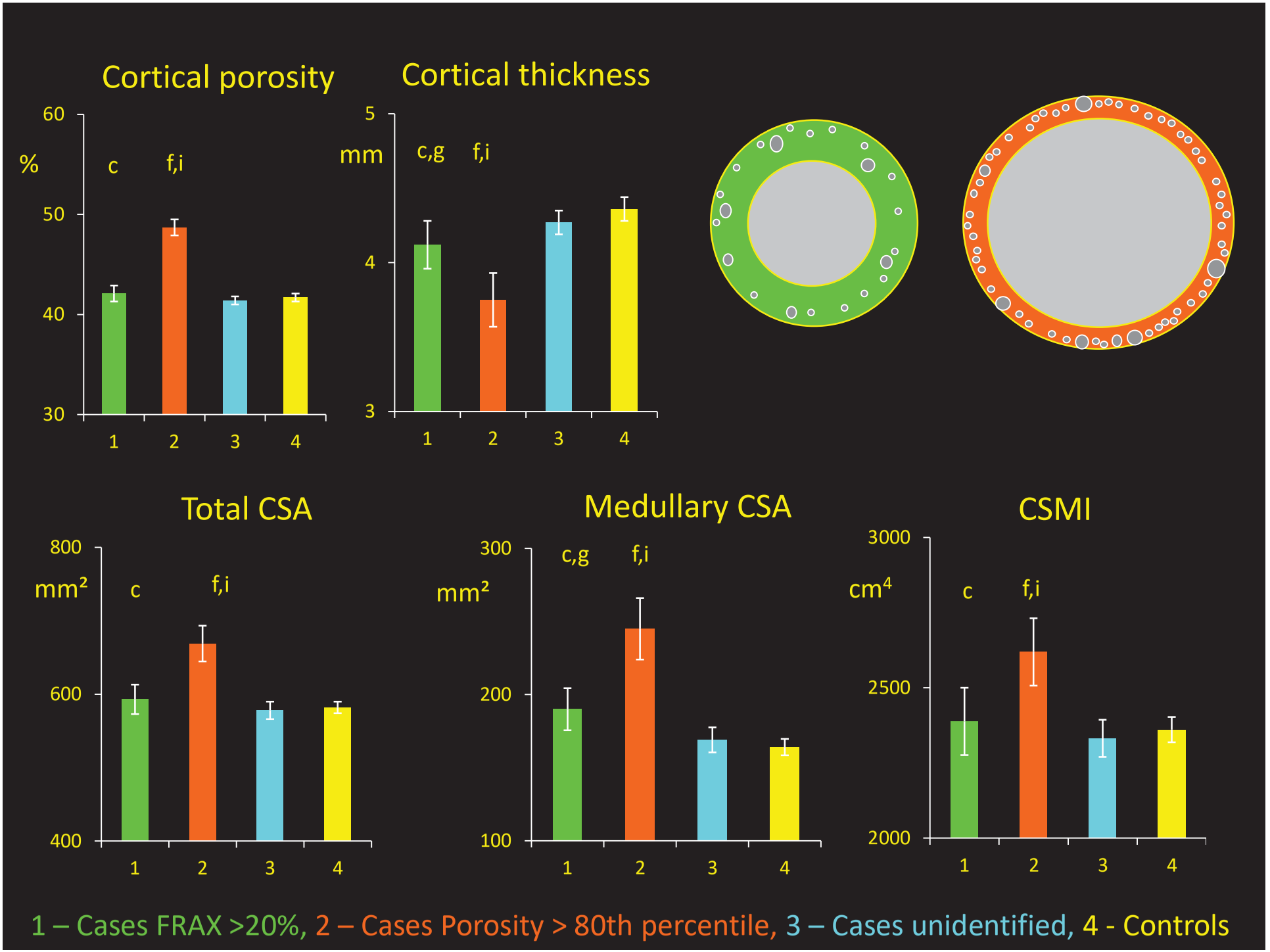
[d]$p < 0.05$, [e]$p < 0.01$, [f]$p < 0.001$ compared to group 3,

[g]$p < 0.05$, [h]$p < 0.01$, [i]$p < 0.001$ compared to group 4.


Compact-appearing cortex
Outer transitional zone
Inner transitional zone
Total Cortex
Marrow cavity


All Fracture cases
n = 211 (100%)
Cases identified by Porosity
n = 61
(28.9%)
Cases identified by FRAX
n = 53
(25.1%)
Group 2
n = 43
(20.4%)
n = 18
(8.5%)
Group 1
n = 35
(16.6%)
Cases not identified by any of these methods (Group 3)
n = 115 (54.5%)


FRAX
%
30
20
10
0
c,f,i
1
2
3
4
Age
years
80
70
60
50
a,f,h
1
2
3
4
FN aBMD
mg/cm²
900
800
700
600
b,f,i
i
i
1
2
3
4
Prior fracture
%
90
60
30
0
c,f
1
2
3
4
1 – Cases FRAX >20%, 2 – Cases Porosity > 80th percentile, 3 – Cases unidentified, 4 - Controls


Cortical porosity
60
%
50
40
30
c
f,i
1
2
3
4
Cortical thickness
5
mm
4
3
c,g
f,i
1
2
3
4
Total CSA
800
mm²
600
400
c
f,i
1
2
3
4
Medullary CSA
300
mm²
200
100
c,g
f,i
1
2
3
4
CSMI
3000
cm⁴
2500
2000
c
f,i
1
2
3
4
1 – Cases FRAX >20%, 2 – Cases Porosity > 80th percentile, 3 – Cases unidentified, 4 - Controls

Paper III

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Full Length Article

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☆

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ABSTRACT

Increased cortical porosity has been suggested as a possible factor increasing fracture propensity in patients with type 2 diabetes mellitus (T2DM). This is a paradox because cortical porosity is generally associated with high bone turnover, while bone turnover is reduced in patients with T2DM. We therefore wanted to test the hypothesis that women with T2DM have lower bone turnover markers (BTM) and lower cortical porosity than those without diabetes, and that higher serum glucose and body mass index (BMI) are associated with lower BTM, and with lower cortical porosity.

This cross-sectional study is based on a prior nested case-control study including 443 postmenopausal women aged 54–94 years from the Tromsø Study, 211 with non-vertebral fracture and 232 fracture-free controls. Of those 443 participants, 22 women exhibited T2DM and 421 women did not have diabetes. All had fasting blood samples assayed for procollagen type I N-terminal propeptide (PINP), C-terminal cross-linking telopeptide of type I collagen (CTX) and glucose, and femoral subtrochanteric architecture was quantified using low-resolution clinical CT and StrAx1.0 software.

Women with T2DM had higher serum glucose (7.2 vs. 5.3 mmol/L), BMI (29.0 vs. 26.4 kg/m^2), and higher femoral subtrochanteric total volumetric bone mineral density (vBMD) (783 vs. 715 mg HA/cm^3), but lower cortical porosity (40.9 vs. 42.8%) than nondiabetic women (all $p < 0.05$). Each standard deviation (SD) increment in glucose was associated with 0.10–0.12 SD lower PINP and CTX, and 0.13 SD lower cortical porosity (all $p < 0.05$). Each SD increment in BMI was associated with 0.10–0.18 SD lower serum PINP and CTX, and 0.19 SD thicker cortices (all $p < 0.05$).

Increasing glucose and BMI were associated with lower bone turnover suggesting that reduced intracortical and endocortical remodeling leads to reduced porosity and thicker cortices. Using low-resolution clinical CT, cortical porosity was lower in women with T2DM compared to women without diabetes. This indicates that other changes in bone qualities, not increased cortical porosity, are likely to explain the increased fracture propensity in patients with T2DM.

1. Introduction

Type 2 diabetes mellitus (T2DM) and bone fragility are public health problems coexisting with increasing age [1,2]. The prevalence of both conditions have increased over the last years, and thus the burden on society [3,4]. Patients with T2DM tend to have higher body mass index

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8756-3282/

(BMI) and areal bone mineral density (aBMD), and would therefore be expected to exhibit reduced risk of fracture. Despite normal or increased aBMD they show increased risk of any fragility fracture [5–8]. T2DM itself is a modest risk factor for fracture, however, given the large number of individuals with this disease, fracture remains a major clinical concern [9].

In T2DM patients, fracture risk is higher for a given level of BMD compared to individuals without diabetes [8]. The reasons for the increased risk for fracture in T2DM patients are not clear [6,10]. Increased cortical porosity of distal radius or distal tibia has been invoked as one possible factor [11–13]. However, it is hard to explain how individuals with T2DM can exhibit high porosity [14–17], since increased cortical porosity reflects increased bone turnover from intracortical surfaces lining the Haversian canals and the endocortical surfaces adjacent to the marrow cavity [18,19]. As T2DM is a condition with low bone turnover [20,21], patients with T2DM would rather be expected to exhibit reduced cortical porosity. In contrast, prior studies reported increased porosity in T2DM patients. However, the absolute differences in cortical porosity were small (0.8–2.4%), they included small sample sizes and measured porosity at peripheral sites using high-resolution peripheral quantitative computed tomography (HR-pQCT) [11–13].

As cortical porosity at peripheral sites may not be representative of central sites, we examined cortical porosity at the proximal femur, a common site of the most serious fragility fracture [22]. Studies using HR-pQCT to quantify porosity present low values of porosity ranging from 1% to 15% because of quantifying only porosity of the compact cortex and only pores over 100 μm [11–13,16], although 60% of cortical pores are under 100 μm in diameter [6,23–25]. This threshold-based image analysis underestimates porosity by including only empty voxels [24]. StrAx1.0 software quantifies porosity as a fraction of void regardless of size of the pores, and captures indirectly porosity produced by pores larger and smaller than 100 μm in diameter. It accounts for partial volume effect by including not only void within total empty voxels, but also partly empty voxels [24]. By using StrAx1.0 software we quantify porosity not only of the compact cortex but also the transitional zone. It is thus more inclusive than the traditional HR-pQCT measurements and the porosity is higher than reported using other methods [24,26].

Common characteristics of patients with T2DM are hyperglycemia and obesity. Increased BMI is associated with reduced bone turnover markers (BTM) and increased aBMD [17,18]. Similar observations with glucose-loading have been made in healthy subjects [15]. However, it remains to be determined whether the serum levels of glucose, BMI, or both, influences cortical porosity, cortical thickness or other bone features. We therefore wanted to test the hypothesis that 1) postmenopausal women with T2DM have lower bone turnover markers and lower cortical porosity than those without diabetes, and that 2) higher serum levels of glucose and BMI are associated with lower bone turnover markers, and with lower cortical porosity.

2. Materials and methods

2.1. Study population

The Tromsø Study is a single-center population-based study in Northern Norway, which conducted six surveys from 1974 to 2008 [27]. In 1994–95 (Tromsø 4), all 37,558 eligible inhabitants in Tromsø over 24 years of age were invited, and 27,158 subjects (72%) participated. In the Tromsø 4 participants, all non-vertebral fractures were registered from the X-ray archives of the University Hospital of North Norway between 1 January 1994 and 1 January 2010 [28]. In 2011, we designed a nested case–control study that included 264 postmenopausal women from the fracture registry, who had suffered at least one fracture of the hip, wrist, or proximal humerus after the age of 50, and 260 age-matched fracture-free controls selected randomly from the same Tromsø 4 cohort, previously described in detail [18,22,29]. Women who were premenopausal, received bisphosphonates for osteoporosis, had hip prostheses, metal screws, or pathological fractures were excluded. Of those 524 participants, we further excluded 15 currently receiving hormone replacement therapy and 66 with movement artifacts during CT scanning. The current study is a cross-sectional study based on the nested case-controls study described above, that included 443 participants (211 fracture cases [4 hip, 181 wrist, and 26 proximal humerus] and 232 fracture-free controls), of those 22 had T2DM and 421 did not have diabetes. The median time since their fracture was 6.6 years (range 1–25). All participants provided written informed consent and all measurements were performed between November 2011 through January 2013. The study was approved by the Regional Committee of Research Ethics and was conducted in accordance with the World Medical Association Declaration of Helsinki.

2.2. Variables

A questionnaire included information concerning fractures after the age of 50 years, number of falls in the last 12 months, diseases, use of medication and lifestyle-factors such as exercise and smoking. The self-reported diagnosis and duration of T2DM was confirmed based on information in their medical records, and diabetic complications were also identified. None of the participants reported that they had type 1 diabetes mellitus. Height and weight were measured in light clothing without shoes, and BMI was calculated as weight/height^2. Total hip and femoral neck (FN) aBMD was measured at the non-dominant side using DXA (GE Lunar Prodigy, Lunar Corporation, Madison, WI, USA), and coefficients of variation (CV) were 1.2% and 1.7%, respectively [22].

Fasting blood samples were collected between 8 and 10 am and assayed for serum glucose using Roche Diagnostics, Germany, with CV 0.5–1.6%, insulin using Elecsys 2010 Modular Analytics E170, Roche Diagnostics, Germany, with CV 0.8–4.6%, 25-hydroxyvitamin D (25(OH)D) using mass spectrometry, parathyroid hormone (PTH) using Immulite 2000, and procollagen type I N-terminal propeptide (PINP) and C-terminal cross-linking telopeptide of type I collagen (CTX) using electrochemiluminescence immunoassay, Elecsys 1010 Analytics, Roche Diagnostics, Germany, with CV of 3–8%. Homeostatic model assessment of Insulin Resistance (HOMA-IR) was calculated using the following formula: (glucose multiplied by insulin) divided by 135 [30], and kidney function was assessed using estimated glomerular filtration rate (eGFR).

CT scans (Siemens Somatom Sensation 16, Erlangen, Germany) of the non-dominant hip were performed at the Department of Radiology, University Hospital of North Norway. The CT machine had an in-plane resolution of 0.74 mm, the slice thickness was 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 [22]. CT scans of the hip were performed at 120 kV, a pitch of 0.75, using 90 mA, reconstructed using a fixed field of view at 120 mm [31]. Quality control was carried out by scanning a phantom containing rods of HA (QRM Quality Assurance in Radiology and Medicine GmbH, Moehrendorf, Germany). These low-resolution CT images were analyzed in Melbourne (Australia) using StrAx1.0 software (StraxCorp Pty Ltd., Melbourne, Australia), a non-thresholding method, which automatically selects attenuation profile curves and segments the bone into compact-appearing cortex, inner and outer transitional zones (TZ), and trabecular compartment (Fig. 1) [32]. This is performed similarly in low-resolution images [22,26,32] as in HR-pQCT images [24,33]. As cortices are thin at the most proximal femur (femoral head, neck and trochanter), analyses were confined to the region of interest (ROI) where the cortices are thicker. This 3.7 mm subtrochanteric region was standardized by starting at tip of lesser trochanter, and the ROI was segmented into its compartments [22,32]. Porosity within each cortical compartment was quantified automatically throughout the ROI using StrAx1.0 software [22,24–26,29,31–33]. StraxCorp was blinded to T2DM and fracture status at the time of the analysis with StrAx1.0. Cortical compartments at the subtrochanteric site are shown in Fig. 1

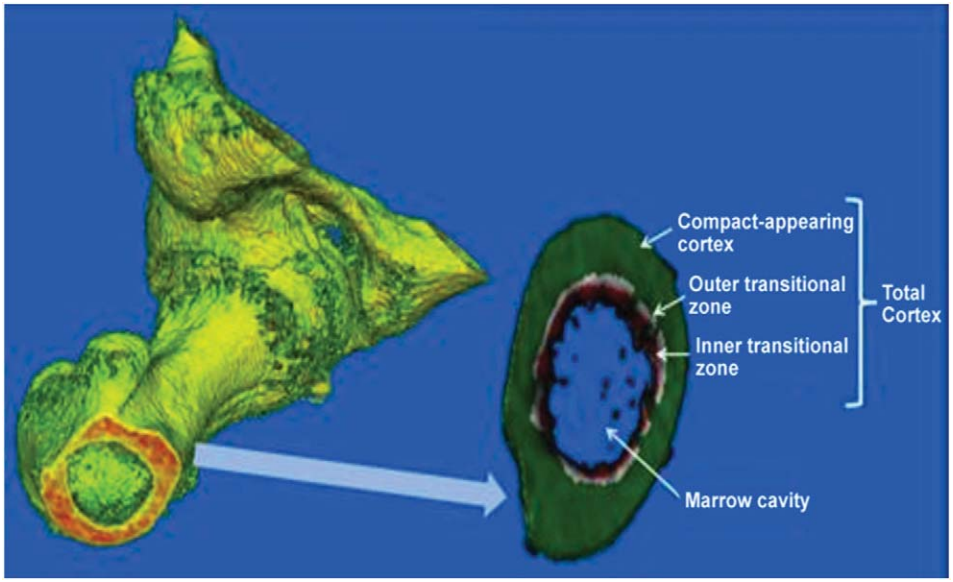

Fig. 1. Cross-section image of proximal femur and its compartments. Segmented computed tomography image obtained at the proximal femur using StrAx1.0, a non-threshold-based segmentation algorithm, showing the total cortex (the area used for the cortical porosity measurements), consisting of the three 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.
(Source: Reproduced with permission of John Wiley and Sons (Ref [32] Zebaze et al. J Bone Miner Res. 31 (2016) 1827–1834).

and prior articles [22,32]. Local bone edges are identified as the beginning and the end of the rising and falling S-shaped portions of the curve enabling the delineation of the compartments [24]. Analyzing ~3600 consecutive overlapping profiles around the perimeter of each cross-sectional slice, segments the compartments. 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 density profile curve is expressing the mineralized bone area as the percentage of total area within each column.

Porosity presented here is defined as and determined as the average void volume fraction within the total cortex (compact appearing cortex, outer and inner transitional zone (TZ)). The porosity quantified by this algorithm is the proportion of emptiness within each voxel or the fraction of the bone occupied by void (porosity) [22,24,32]. StrAx1.0 quantifies porosity in low-resolution CT even though pores are not visible to the naked eye and it is thus an indirect measure of porosity [18,22,26,29,32]. The size and number of pores were not determined by using this software. Accuracy of porosity measurements at distal radius and distal tibia using HR-pQCT images with a voxel size of 82 μm was validated against μCT images of cadaver specimens with a voxel size of 19 μm as the gold standard [24]. Zebaze et al. also assessed accuracy of porosity quantified at the proximal femur in HR-pQCT images, against scanning electron microscopy (SEM) images of specimens collected at 2.5 μm resolution as the gold standard [24]. The agreement (R^2) between HR-pQCT and these gold standards for quantification of porosity ranged from 0.87 to 0.99. The in vivo precision of StrAx1.0 analysis of HR-pQCT images was tested by rescanning seven women four times [24]. The in vivo and ex vivo precision error was <4.0% [24].

Accuracy of porosity measurements using clinical CT images with a voxel size of 740 μm was validated by testing agreement with HR-pQCT measurements with a voxel size of 82 μm as the gold standard [22,32]. The agreement (R^2) between CT and HR-pQCT ranged from 0.86 to 0.96 for quantification of porosity at the same femoral subtrochanteric site (range 40–95%) [22,32]. CV for porosity of each cortical compartment were below 4.0%. For ethical reasons, it was not possible to perform in vivo validation with rescanning of women on the same day. Therefore, we performed an additional validation by repositioning and rescanning a human hip phantom (consisting of a human pelvic skeleton embedded in plastic material) 10 times, with CV for the CT subtrochanteric bone parameters between 0.3 and 2.3% [22]. Thus, StrAx1.0 software provides accurate and reliable measurements of cortical porosity and other bone traits.

2.3. Statistical analyses

In this cross-sectional study, results are presented stratified by T2DM-status and by fracture status. All normally distributed continuous variables are presented as mean ± standard deviation (SD). Remaining variables; trabecular BV/TV, serum insulin and HOMA-IR, are presented as median (range). In order to correct for skewed distribution, we used log-transformed trabecular BV/TV in the analysis. Differences between women with and without T2DM were assessed using analysis of covariance (ANCOVA), adjusted for age and fracture status. In sub-analysis, we compared diabetic women with and without fracture, and nondiabetic women with and without fracture using ANOVA, 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 (STB) were used to facilitate the comparison of the strength of the associations between the exposure and endpoints. Analyses were performed using STATA Software package, v14 (StataCorp, LP, Texas, USA) and SAS software package, v9.3 (SAS Institute Inc., Cary, NC, USA). All tests were two-sided and $p < 0.05$ considered significant.

3. Results

3.1. Women with type 2 diabetes mellitus compared with those without diabetes

Women with T2DM exhibited higher fasting serum levels of glucose (7.2 vs. 5.3 mmol/L) and insulin (102 vs. 55 pmol/L), higher HOMA-IR (5.3 vs. 2.2) and BMI (29.0 vs. 26.4 kg/m^2), and lower serum vitamin D (67.6 vs. 80.4 nmol/L) than those without diabetes (all $p < 0.05$, Table 1). Moreover, they had higher total vBMD (783 vs.

Table 1
Characteristics of women with type 2 diabetes mellitus and controls.

	Type 2 diabetes mellitus Mean ± SD	Controls without diabetes Mean ± SD	p
n	22	421	
Age (years)	70.9 ± 7.2	68.2 ± 7.2	0.089
Weight (kg)	73.8 ± 12.1	69.2 ± 10.6	0.040
Height (cm)	159.5 ± 7.8	162.0 ± 6.3	0.232
Body mass index (kg/m^2)	29.0 ± 4.8	26.4 ± 4.0	0.006
Physical activity (hour/week)	2.5 ± 1.7	2.5 ± 1.6	0.877
Currently smoker, n (%)	2 (9.1)	51 (12.1)	0.892
Prevalent fracture, n (%)	9 (40.9)	202 (48.0)	0.513
Falls in last 12 months, n (%)	7 (31.8)	151 (35.9)	0.651
Self-reported good health, n (%)	11 (50.0)	301 (71.8)	0.045
Take calcium supplements, n (%)	3 (13.6)	69 (16.4)	0.710
Take vitamin D supplements, n (%)	16 (72.7)	313 (74.4)	0.866
Fasting serum glucose (mmol/L)	7.23 ± 2.05	5.33 ± 0.49	<0.001
Fasting serum insulin (pmol/L)[a]	102 (27–1117)	55 (12–397)	<0.001
HOMA-IR[a]	5.3 (1.1–114)	2.2 (0.4–21)	<0.001
Fasting serum PINP (ng/mL)	40.4 ± 13.7	46.8 ± 16.6	0.100
Fasting serum CTX (ng/mL)	0.461 ± 0.264	0.464 ± 0.172	0.988
Serum vitamin D (nmol/L)	67.6 ± 21.2	80.4 ± 24.7	0.012
Serum PTH (pmol/L)	4.98 ± 1.81	4.31 ± 2.14	0.206
eGFR (ml/min)	74.2 ± 21.3	77.8 ± 15.5	0.584
Total hip T-score	0.71 ± 1.04	0.89 ± 1.00	0.343
Total hip aBMD (mg/cm^2)	915 ± 125	893 ± 120	0.343
Femoral neck (FN) aBMD (mg/cm^2)	829 ± 104	829 ± 112	0.869
Femoral subtrochanteric architecture[b]			
Total bone vBMD (mg HA/cm^3)	783 ± 110	715 ± 105	0.002
Cortical vBMD (mg HA/cm^3)	1073 ± 77.3	1041 ± 66.3	0.029
Cortical thickness (mm)	4.25 ± 0.50	4.21 ± 0.58	0.716
Total cortical porosity (%)	40.9 ± 4.39	42.8 ± 3.97	0.029
Compact cortical porosity (%)	33.9 ± 3.49	34.8 ± 2.89	0.185
Outer transitional zone porosity (%)	45.6 ± 2.58	45.4 ± 2.28	0.630
Inner transitional zone porosity (%)	84.0 ± 1.56	84.1 ± 1.50	0.624
Trabecular BV/TV (%)[a]	0.13 (0.04–1.28)	0.17 (0.01–1.82)	0.599

Women with type 2 diabetes mellitus were compared with controls (without diabetes) using ANCOVA, adjusted for age and fracture status. SD, standard deviation; Prevalent fracture (≥1 vs 0); Falls in last 12 months (≥1 vs 0); HOMA-IR, homeostatic model assessment of Insulin Resistance; PINP, procollagen type I N-terminal propeptide; CTX, C-terminal cross-linking telopeptide of type I collagen; PTH, parathyroid hormone; eGFR, estimated glomerular filtration rate, aBMD, areal bone mineral density, vBMD, volumetric bone mineral density; BV/TV, bone volume/tissue volume.

[a] Median (range) are presented due to skewed distribution, otherwise numbers are mean ± SD.

[b] Assessed using low-resolution clinical CT.

715 mg HA/cm^3) and cortical vBMD (1073 vs. 1041 mg HA/cm^3), lower total cortical porosity (40.9 vs. 42.8%) of the femoral subtrochanteric region (all $p < 0.05$). Serum levels of PINP in women with T2DM tended to be lower than in women without diabetes (40.4 vs. 46.8 ng/mL), though this did not reach significance ($p = 0.100$). All results were adjusted for age and fracture status.

3.2. Glucose, insulin, BMI, and bone turnover markers

Each SD increment in serum glucose was associated with 0.12 and 0.10 SD lower serum PINP and CTX, respectively (both $p < 0.05$, Table 2, Fig. 2a). Each SD increment in serum insulin and HOMA-IR were each associated with 0.10 SD lower CTX ($p < 0.05$), but not associated with PINP. Each SD increment in BMI was associated with 0.10 and 0.18 SD lower serum PINP and CTX, respectively (both $p < 0.05$, Table 2, Fig. 2b).

3.3. Glucose, insulin, BMI, cortical porosity, and cortical thickness

Each SD increment in serum glucose, insulin, HOMA-IR and BMI was associated with 0.12–0.19 SD higher total vBMD (all $p < 0.05$). Each SD increment in serum glucose was associated with 0.13 SD lower cortical porosity ($p = 0.006$), while serum insulin and HOMA-IR were not associated with cortical thickness or porosity. Each SD increment in BMI was associated with 0.19 SD thicker cortices ($p < 0.001$). All results were adjusted for age and fracture status. Excluding those with the highest serum glucose above 7 mmol/L or those above 10 mmol/L did not change the results, as glucose remained associated with cortical porosity ($p < 0.05$).

The median duration of T2DM was 6.7 years (range 1.8–17.9), and duration of diabetes was inversely but non-significantly associated with cortical porosity (STB = −0.28, $p = 0.222$, Fig. 3). Of 22 women with T2DM, seven were not on medication for diabetes, 12 used only oral antidiabetics, while three used both oral antidiabetics and insulin. Only three women with T2DM had complications (one neuropathy, two nephropathy), however these three patients had cortical porosity ranging from 38 to 43%, which was close to the average levels of cortical porosity, and they had additionally comorbidity as cancer and hypertension.

3.4. Cortical porosity in women with type 2 diabetes mellitus and fracture

Women with T2DM had lower cortical porosity than those without diabetes ($p = 0.033$), and women with fracture had higher porosity than fracture–free controls ($p < 0.001$, Fig. 4). We stratified the analysis. In women with T2DM, those with fracture had non-significantly higher porosity than those without fracture ($p = 0.159$, Fig. 4, Table 3). In women without T2DM, those with fracture had higher porosity than those without fracture ($p < 0.001$). In women with fracture, those with T2DM had non-significantly lower cortical porosity than those

Table 2
Associations of a 1 SD increment in body mass index (BMI), serum levels of glucose and insulin, and insulin resistance with bone turnover markers and femoral subtrochanteric architecture.

	BMI (kg/m^2)		Glucose (mmol/L)		Insulin (pmol/L)		HOMA-IR	
	STB	p	STB	p	STB	p	STB	p
Bone turnover markers								
PINP (ng/mL)	−0.10	0.040	−0.12	0.012	−0.02	0.658	−0.03	0.546
CTX (ng/mL)	−0.18	<0.001	−0.10	0.030	−0.10	0.042	−0.10	0.039
Femoral subtrochanteric architecture[a]								
Total vBMD (mg HA/cm^3)	0.19	<0.001	0.19	<0.001	0.12	0.007	0.12	0.007
Cortical thickness (mm)	0.19	<0.001	0.08	0.090	0.08	0.086	0.07	0.114
Total cortical porosity (%)	−0.03	0.503	−0.13	0.006	−0.06	0.207	−0.08	0.077
Trabecular BV/TV[b]	0.12	0.010	0.06	0.177	0.07	0.138	0.06	0.235

SD, standard deviation; HOMA-IR, homeostatic model assessment of Insulin Resistance; PINP, procollagen type I N-terminal propeptide; CTX, C-terminal cross-linking telopeptide of type I collagen; vBMD, volumetric bone mineral density; BV/TV, bone volume/tissue volume. STB, standardized beta coefficients using linear regression analyses and adjusted for age and fracture status.

[a] Assessed using low-resolution clinical CT.

[b] Analyzed using log-transformed variables.

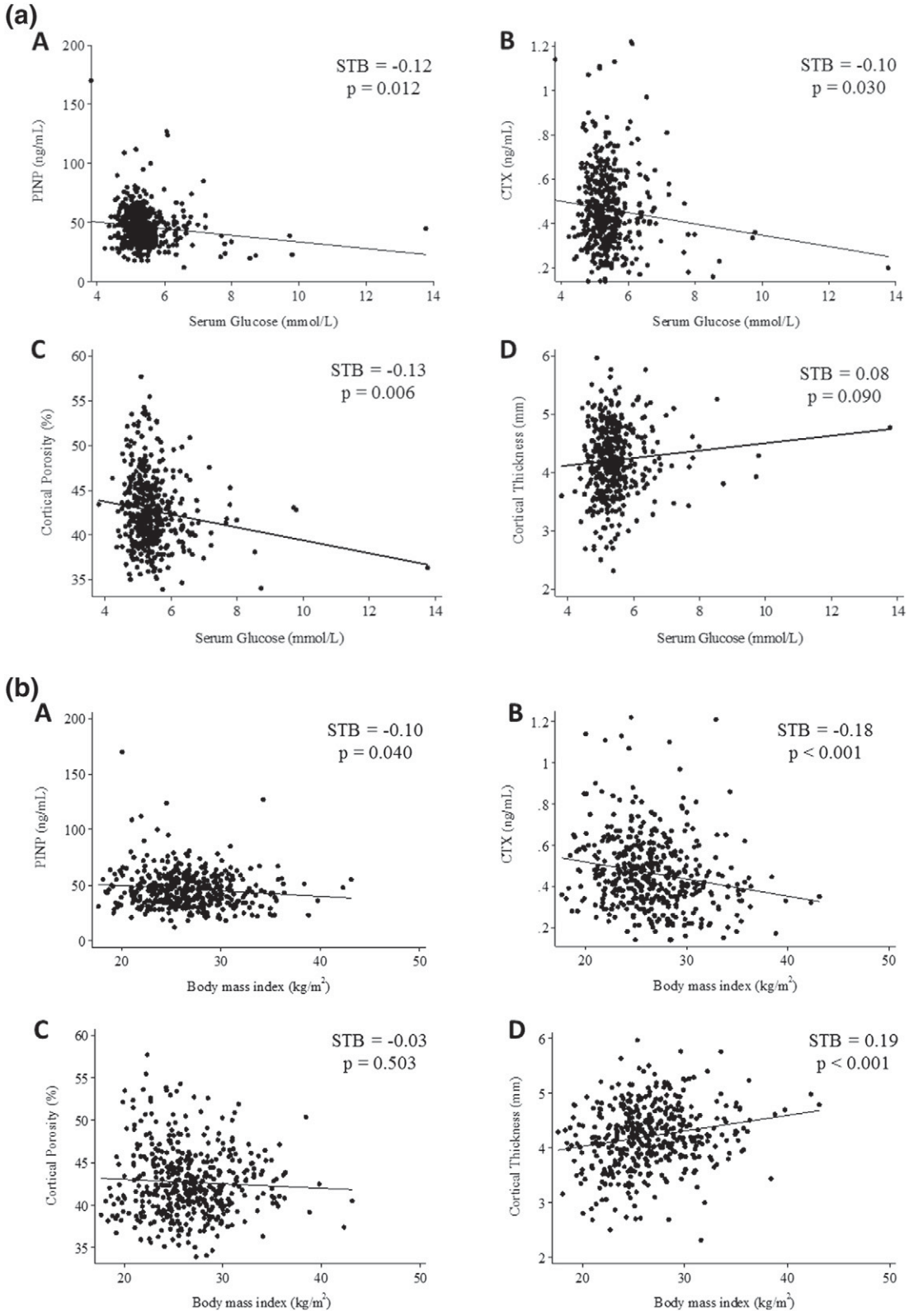

Fig. 2. a. (A) Procollagen type I N-terminal propeptide (PINP), (B) C-terminal cross-linking telopeptide of type I collagen (CTX), and (C) femoral subtrochanteric porosity of the total cortex (compact-appearing cortex, inner and outer transitional zones) and (D) cortical thickness as a function of serum glucose. The standardized beta coefficients (STB) are estimated using linear regression analyses and adjusted for age and fracture status. Bone architecture was assessed using low-resolution clinical CT. b. (A) Procollagen type I N-terminal propeptide (PINP), (B) C-terminal cross-linking telopeptide of type I collagen (CTX), and (C) femoral subtrochanteric porosity of the total cortex (compact-appearing cortex, inner and outer transitional zones) and (D) cortical thickness as a function of body mass index (BMI). The standardized beta coefficients (STB) are estimated using linear regression analyses and adjusted for age and fracture status. Bone architecture was assessed using low-resolution clinical CT.

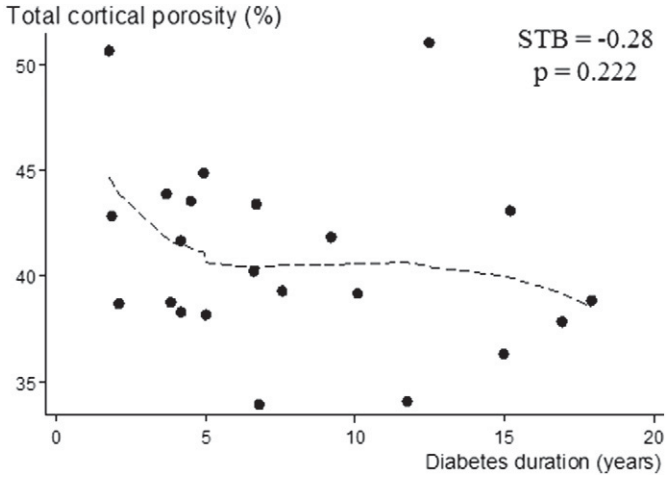

Fig. 3. Femoral subtrochanteric porosity of the total cortex (compact-appearing cortex, inner and outer transitional zones) assessed using low-resolution clinical CT as a function of diabetes duration. The standardized beta coefficients (STB) are estimated using linear regression analyses and adjusted for age, body mass index and fracture status.

without T2DM ($p = 0.385$). In women without fracture, those with T2DM had lower porosity than those without T2DM ($p = 0.048$). All analysis were adjusted for age and BMI.

In stratified analysis of women without T2DM, those with fracture were taller, had lower BMI, higher serum PINP and CTX, lower vitamin D, higher PTH, lower aBMD of the total hip and femoral neck, lower total and cortical vBMD, and thinner cortices of femoral subtrochanteric site than those without fracture (all $p < 0.05$, Table 3). In stratified analysis of women with T2DM, there were similar trends, although few associations achieved statistical significance.

4. Discussion

We report that women with T2DM exhibited lower cortical porosity of the femoral subtrochanteric region than nondiabetic women. This was quantified using low-resolution clinical CT. Women with T2DM had higher total vBMD and cortical vBMD than nondiabetic women. The bone formation marker PINP tended to be lower in those with T2DM than nondiabetic women but did not reach statistical significance. Women with T2DM had higher serum glucose, insulin, and BMI compared to nondiabetic women, and increasing glucose and BMI were associated with lower PINP and CTX. Increasing glucose was associated with reduced cortical porosity, whereas increasing BMI was associated with thicker cortices, thus it seems that both increased glucose levels and BMI contributed to preserving the architecture of the cortical bone.

The reasons why patients with T2DM have increased risk of fracture, despite normal BMD are still poorly elucidated. Higher cortical porosity has been invoked as a possible factor increasing fracture propensity [11–13,16]. In a pilot study, 19 postmenopausal women with T2DM had higher cortical porosity at the distal radius than 19 controls (4.3% vs. 1.9%) [13]. However, this difference in porosity was reduced after exclusion of two fracture patients with T2DM who had 2-fold greater porosity than the group mean, and the authors suggested that the high porosity in the T2DM group could be attributed to the contribution of the fracture subjects [13]. In another study, 22 African-American women with T2DM exhibited higher cortical porosity of the distal radius than 78 nondiabetic controls (2.9% vs. 2.3%) [12]. Postmenopausal women with T2DM of >10 years duration, had a non-significant tendency towards higher radial cortical porosity compared to controls (3.0% vs 2.2%) [16], while 11 older women (72–81 years) with diabetes had higher radial porosity than 144 nondiabetic controls (4.3% vs 3.4%) [11]. These data suggest a tendency towards increased cortical porosity of the distal radius in women with T2DM in studies with limited number of participants using HR-pQCT. However, the absolute differences in cortical porosity between those with and without T2DM were small, and the cortical thickness was below 1 mm at the distal radius [11–13,34]. Moreover, HR-pQCT present low values of porosity (1–15%) because of quantifying only porosity of the compact cortices and only pores over 100 μm in diameter, and the threshold based segmentation may misclassify trabecularised cortical bone as trabecular bone [23,24,26,32].

In contrast to previous reports on higher cortical porosity of distal radius using HR-pQCT, we report lower cortical porosity of the proximal femoral shaft using low-resolution CT, in a small sample of 22 women with T2DM than in nondiabetic women. The large control group of 421 nondiabetic women added statistical power to the analysis. These results remained unchanged after exclusion of outliers with the highest levels of glucose. Moreover, in a recent study using HR-pQCT for assessment of microarchitecture, the distal radial cortical porosity was lower

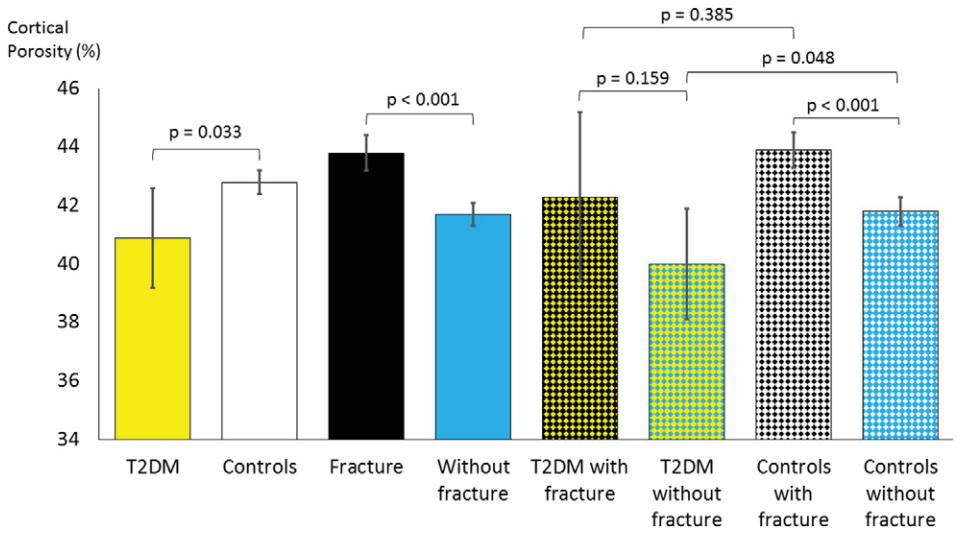

Fig. 4. Mean porosity with 95% confidence intervals (±1.96 standard errors of the mean) of the total cortex (compact-appearing cortex, inner and outer transitional zones) in women with type 2 diabetes mellitus (T2DM) (yellow, n = 22) and controls (without diabetes) (white, n = 421), in women with fracture (black, n = 211) and without fracture (blue, n = 232). Porosity in stratified analysis, in women with T2DM with fracture (n = 9), in those with T2DM without fracture (n = 13), in controls with fracture (n = 202) and controls without fracture (n = 219). All comparisons of groups were adjusted for age and body mass index. Porosity was assessed using low-resolution clinical CT.

Table 3
Characteristics of women with type 2 diabetes mellitus and controls stratified by fracture status.

	Type 2 diabetes mellitus		Controls	
	With fracture	Without fracture	With fracture	Without fracture
n	9	13	202	219
Age (years)	69.2 ± 7.1	72.1 ± 7.3	68.4 ± 7.8	68.1 ± 6.7
Weight (kg)	74.1 ± 11.6	73.5 ± 12.9	68.6 ± 10.5	69.8 ± 10.7
Height (cm)	160.8 ± 10.9	158.7 ± 5.0	162.7 ± 5.8	161.4 ± 6.7[a]
Body mass index (BMI) (kg/cm^2)	28.8 ± 4.5	29.2 ± 5.1	25.9 ± 3.7	26.8 ± 4.2[a]
Physical activity (hours/week)	2.8 ± 1.9	2.3 ± 1.6	2.6 ± 1.6	2.5 ± 1.7
Fasting serum glucose (mmol/L)	7.19 ± 2.91	7.27 ± 1.30	5.29 ± 0.47	5.38 ± 0.51
Fasting serum insulin (pmol/L)[d]	103 (32–1117)	99 (27–160)	53 (12–316)	57 (14–397)
HOMA-IR[d]	5.2 (1.1–114)	5.4 (1.3–11.6)	2.1 (0.4–15)	2.2 (0.5–21)
Serum PINP (ng/ml)	48.2 ± 9.26	35.0 ± 14.0[a]	49.7 ± 18.5	44.0 ± 14.0[c]
Serum CTX (ng/ml)	0.46 ± 0.18	0.47 ± 0.32	0.49 ± 0.19	0.44 ± 0.16[a]
Serum Vitamin D (nmol/L)	61.2 ± 21.6	72.1 ± 20.6	77.1 ± 22.4	83.5 ± 26.3[b]
Serum PTH (pmol/L)	4.76 ± 1.75	5.13 ± 1.90	4.57 ± 2.43	4.07 ± 1.80[a]
eGFR (ml/min)	68.4 ± 15.3	78.3 ± 24.4	77.8 ± 16.8	77.8 ± 14.2
Total hip aBMD (mg/cm^2)	864 ± 111	950 ± 126[a]	853 ± 114	930 ± 115[c]
Femoral neck (FN) aBMD (mg/cm^2)	798 ± 73.6	851 ± 119[a]	794 ± 101	861 ± 112[c]
Femoral subtrochanteric architecture[e]				
Total bone vBMD (mg HA/cm^3)	758 ± 143	800 ± 83.4	681 ± 110	747 ± 89.7[c]
Cortical vBMD (mg HA/cm^3)	1050 ± 98.9	1088 ± 47.4	1024 ± 71.3	1057 ± 56.8[c]
Cortical thickness (mm)	3.97 ± 0.41	4.44 ± 0.47[a]	4.06 ± 0.58	4.35 ± 0.55[c]
Total cortical porosity (%)	42.3 ± 5.92	40.0 ± 2.84	43.9 ± 4.27	41.8 ± 3.40[c]

In women with type 2 diabetes mellitus, those with and without fracture were compared, and similarly in controls, women with and without fracture were compared using ANCOVA adjusted for age and BMI. HOMA-IR, homeostatic model assessment of Insulin Resistance; PINP, procollagen type I N-terminal propeptide; CTX, C-terminal cross-linking telopeptide of type I collagen; PTH, parathyroid hormone; eGFR, estimated glomerular filtration rate; aBMD, areal bone mineral density; vBMD, volumetric bone mineral density; BV/TV, bone volume/tissue volume.

[a] $p < 0.05$.
[b] $p < 0.01$.
[c] $p < 0.001$.
[d] Median (range) are presented due to skewed distribution, otherwise numbers are mean ± standard deviation.
[e] Using low-resolution clinical CT.

in 99 women with T2DM than in 316 nondiabetic women (1.5% vs 2.0%, $p = 0.001$) [35]. Thus, women with T2DM have lower porosity when measured at a peripheral site as well as a central site, and by using HR-pQCT as well as low resolution CT imaging technology.

Patsch et al. reported higher cortical porosity of the distal radius and tibia in women with T2DM and fragility fractures compared to those with T2DM without fractures, but a non-significant tendency of lower cortical porosity at most sites when comparing diabetic to nondiabetic women [36]. The higher cortical porosity in women with T2DM with fracture than T2DM without fracture, is similar to prior reports of higher porosity in fracture cases than fracture-free controls [33,37,38]. Patsch et al. did not report higher porosity in patients with T2DM than in those without T2DM. In fact, they reported that T2DM patients without fracture had the lowest cortical porosity of all groups, and they had a non-significantly lower porosity than non-diabetic women. In agreement with Patsch et al., we report that women with fracture had higher porosity than those without fracture, while women with T2DM had lower porosity than nondiabetic women. Moreover, cortical porosity is suggested to be increased only in T2DM patients with microvascular complications, however, one limitation in that study was that fracture status was not taken into account [34]. They reported no correlation between bone structure and duration of disease, which is in agreement with our findings of a non-significant association between cortical porosity and diabetes duration. Nevertheless, taking together findings from prior studies and the current study, robust evidence for higher or lower cortical porosity in larger groups of patients with T2DM than in nondiabetic controls, is still lacking.

We used CT and StrAx1.0 software for quantification of bone architecture [22]. StrAx1.0 is a non-threshold based algorithm, which takes the transitional zone into account and is thus more inclusive than HR-pQCT. This is important because there is a gradual change in attenuation from the outer to the inner part of the bone, and it is a challenge to separate cortical from trabecular bone accurately [24]. By recognizing the transitional zone, we avoid misclassification of trabecularised cortex as trabecular bone [23,25]. Moreover, the StrAx1.0 algorithm does not quantify the number or size of pores within the cortex, but the fraction of void volume within the total cortical bone volume. Thus the partial volume effect is taken into account by including porosity in voxels containing both void volume and bone matrix, regardless of the pore size [23,24]. Studies using threshold-based methods for assessment of cortical porosity present lower values of porosity (between 1% and 15%) because of quantifying only porosity of the compact-appearing cortex and only pores above 100 μm in diameter [6,24]. This again may partly explain the differences in the results reported in the current study compared to previous studies of cortical porosity in patients with T2DM.

As high bone turnover is reported to be associated with increased cortical porosity [18,19], it is hard to explain how patients with T2DM can have low bone turnover and increased cortical porosity [11–13,16,36]. Previous reports have been consistent, and reported lower levels of BTM in subjects with T2DM compared to nondiabetic subjects [6,16,21,39]. In insulin resistant [40], obese [17], and healthy subjects [15], glucose-loading is reported to exhibit a suppressive effect on BTM. An oral glucose tolerance test suppressed PINP and CTX by two-fourfold, higher fasting serum levels of glucose were associated with lower PINP, and higher levels of insulin were associated with increased bone formation markers [15]. We confirmed that higher glucose levels and higher BMI were associated with lower serum PINP and CTX.

In the current study, higher glucose was associated with lower cortical porosity, suggesting glucose to be involved in the pathophysiological mechanisms of changes in cortical bone. This association is novel, but weak, and needs to be confirmed in larger studies. We did not measure glycosylated hemoglobin and glucose does not necessarily reflect the long-term hyperglycemia. However, glucose was measured at a standardized setting in fasting morning samples. BMI was not associated with lower cortical porosity, but with thicker cortices. As there is a tight association between obesity, insulin resistance and T2DM development this is not surprising, as thicker tibial cortices and lower tibial cortical porosity is reported in obese women [41]. Generally, obesity has been considered protective against fractures, but studies in the last decade have shown that obese individuals have greater risk of

several types of fracture [42]. When adjusted for BMD, increasing BMI is protective for hip fracture only [43,44].

What is the reason for increased fracture propensity in T2DM, if not increased cortical porosity? Although low bone turnover rate, as reflected by low BTM, is associated with reduced risk of fracture, there might be both advantages and disadvantages to low bone turnover. As an example, patients treated over several years with anti-resorptive agents who suffer an atypical femoral fracture, are suggested to have reduced cortical porosity and more mineralized bone matrix at the distal radius [45]. Bone qualities and strength are dependent on many material and structural features, as the collagen cross-linking, mineralization and collagen content [15,46]. In patients with T2DM, cross-linking of collagen and secondary mineralization might affect bone qualities negatively, despite of low bone remodeling that might slow loss of bone [15,47]. Another possible explanation of deteriorated bone quality in T2DM, is accumulation of advanced glycation end products (AGEs) in the bone matrix because of high glucose levels, giving increased brittleness of collagen, and thereby microdamage [4,15]. It is also well established that AGEs increase with age and that glycation is associated with altered osteoblast activity [9], and high glucose in vitro might increase apoptosis of osteoblasts [15,48].

The reasons for skeletal fragility in T2DM are not completely understood, and the pathogenesis of the bone fragility in T2DM is likely multifactorial. In addition to insulin resistance, and hyperglycemia with accumulation of AGEs and subsequent impaired bone material properties to brittleness of collagen, other factors may be important. Obesity with accompanying increased visceral fat, and oxidative stress can lead to altered structure and strength of bone, and late stages of diabetes with failure of β cell as well as vascular complications. Both obesity and chronic hyperglycemia can lead to changes in the osteoblast receptor signaling [6]. For patients with T2DM, the diabetic complications as neuropathy and retinopathy might cause falls, leading to fracture [6]. However, a large study of 124,655 fracture cases and 373,962 controls reported that diabetes in general carry an increased fracture risk, but diabetic complications did not explain the overall fracture risk [49]. Women with T2DM still exhibited greater risk for fracture after adjustment for impaired vision and fall [50]. In the current study, women with T2DM did not report more falls in the last 12 months than did nondiabetic women. Moreover, duration of T2DM was not associated with higher cortical porosity in the present study, but in fact a non-significant trend of lower cortical porosity.

The strength of this study is that it is based on a general population, with high participation rate, [27], and the diagnosis of T2DM was confirmed based on medical records. The StrAx1.0 software for quantification of bone architecture is validated by confirming strong correlation between measurements using CT and HR-pQCT, and also by rescanning a human hip phantom using the same CT machines as used for the participants of the study with good reproducibility [22]. The measurements were obtained from the femoral subtrochanteric region, a central site that consists of a thicker cortex so that cortical architecture could be well quantified.

The limited number of T2DM patients in this study is a limitation, however, the large group of controls adds strength to the statistical analysis. The retrospective case-control design may have introduced selection bias, so it is possible that the strength of the associations is somewhat underestimated due to a "healthy" selection bias. Information from the self-administered questionnaires might be prone to recall bias, resulting in over- or under-reporting. Furthermore, the subtrochanteric site contained little trabecular bone, so the possibility to test its association with diabetes or other factors was limited.

In conclusion, women with type 2 diabetes mellitus had lower cortical porosity of the femoral shaft than nondiabetic women, assessed using low-resolution CT and StrAx1.0 software. Cortical porosity assessed using this software includes porosity not only of the compact cortex, but also the transitional zone, and is thus more inclusive than other methods. Increasing glucose and BMI were associated with reduced bone turnover markers. Reduced bone turnover will lead to reduced intracortical and endocortical remodeling and reduced porosity of thicker cortices. Such changes in bone features would be expected to reduce fracture risk. Increasing cortical porosity is thus unlikely to explain the increased fracture propensity in women with type 2 diabetes. Therefore, other factors as alterations in bone material composition or increased microdamage due to reduced bone turnover are more likely contributors to reduced bone qualities and increased fracture risk in these patients.

Disclosures

EFE reported that he has received lecture fees from Amgen, Novartis, Eli Lilly and IDS. All authors state that they have no other conflict of interest.

Authors' roles

Study concept, design, obtained funding and executed the study: MO, RK, ÅB. Statistical analysis: MO, ÅB. Drafting manuscript: MO, RK, TTB, IKH, RMJ, EFE, ÅB. Data interpretation and critical revision of the manuscript for important intellectual content, writing of the report and approval of the final version: MO, RK, TTB, IKH, RMJ, EFE, ÅB. ÅB takes responsibility for the integrity of the data analyses.

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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

01-2011 Beinstruktur
01.11.2011

Dato ☐☐.☐☐.2☐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

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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

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

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!

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