UiT
THE ARCTIC
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OF NORWAY

Faculty of Health Sciences,

Department of Community Medicine

Cortical porosity, medullary adiposity, type 2 diabetes mellitus, serum vitamin D, parathyroid hormone, and nonvertebral fractures

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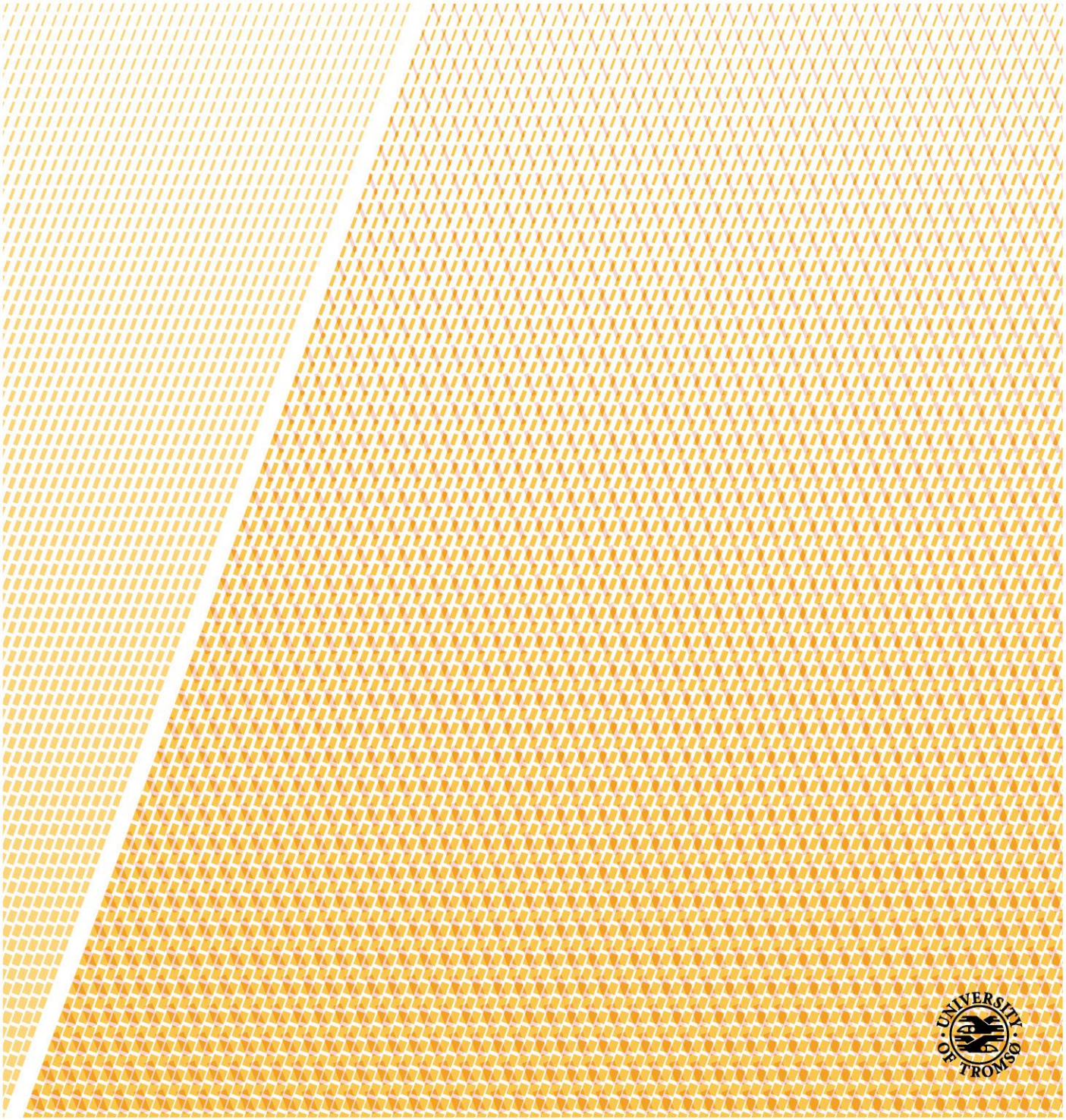
Marit Osima

A dissertation for the degree of Philosophiae Doctor, February 2018



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UNIVERSITY HOSPITAL OF NORTH NORWAY
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Tromsø, February 2018

Marit Osima

Summary

Background

We studied risk factors for fracture and explored the role of cortical porosity in the associations between risk factors and nonvertebral fracture. We tested the hypotheses that (i) higher medullary adiposity is associated with increased cortical porosity and fewer trabeculae, (ii) medullary adiposity and cortical porosity are each independently associated with prevalent nonvertebral fracture, and identifying more women with fractures than either trait alone, (iii) postmenopausal women with type 2 diabetes mellitus (T2DM) have lower bone turnover markers (BTM) and lower cortical porosity than those without diabetes, (iv) higher serum glucose and body mass index (BMI) are associated with lower BTM and lower cortical porosity, (v) serum 25-hydroxyvitamin D (25(OH)D) and parathyroid hormone (PTH) are associated with cortical porosity, and (vi) associations of 25(OH)D) and PTH with fracture risk are dependent on cortical porosity.

Methods

Paper I: In a case-control study, 79 women aged 40-70 years with nonvertebral fractures and 345 controls were recruited in Melbourne, Australia. All women had distal tibia and distal radius medullary adiposity, and cortical and trabecular microarchitecture quantified, using high-resolution peripheral quantitative computed tomography (HR-pQCT).

Papers II and III: In a nested case-control study, we included 211 postmenopausal women aged 54-94 years with nonvertebral fractures and 232 controls from the Tromsø Study. In pooled data, analyzed as a single cohort, 22 of these women had T2DM and 421 women did not have diabetes. Serum 25(OH)D, PTH and BTM were measured, and femoral subtrochanteric architecture was quantified using low-resolution CT images.

Results

Paper I: Tibial medullary adiposity and cortical porosity were associated with increased odds ratio (OR) for fracture (OR [95% confidence interval] 3.43 [2.24-5.27] and 1.88 [1.23-2.85]), adjusted for age and femoral neck areal bone mineral density. Of 77 women with fracture, a medullary adiposity threshold >80th percentile identified 22 women (28.6%) with fracture missed by cortical porosity, while cortical porosity threshold >80th percentile identified 11 women (14.3%) missed by medullary adiposity. Sensitivity was 52.0% using only medullary adiposity, 37.7% using only cortical porosity, and 66.2% using both, and specificity using both was 77.3%. Results were similar for the distal radius.

Paper II: Women with T2DM had lower cortical porosity than those without, higher glucose was associated with lower BTM and lower cortical porosity. Higher BMI was associated with lower BTM and thicker cortices.

Paper III: Women with fracture had lower serum 25(OH)D and higher PTH and BTM than controls, and they had increased femoral subtrochanteric cortical porosity, and reduced cortical thickness. Lower serum 25(OH)D was not associated with cortical parameters or BTM. Higher PTH was associated with increased BTM and higher cortical porosity of the inner transitional zone. Moreover, decreasing 25(OH)D and increasing PTH increased odds for fracture independent of cortical porosity and covariates.

Conclusions

Combining medullary adiposity and cortical porosity may improve identification of women at risk for fracture. Cortical porosity is lower in women with T2DM than in those without. PTH increases intracortical bone turnover, leading to trabecularization of the inner cortical bone.

List of papers

The thesis is based on the following papers:

Paper I

Osima M, Zebaze R, Bui M, Lukic M, Wang X, Ghasem-Zadeh A, Eriksen EF, Seeman E, Bjørnerem Å. Combining medullary adiposity and cortical porosity identifies more women with nonvertebral fractures than either measurement alone. Submitted to J Bone Miner Res, 2017; Dec 30. Under review.

Paper II

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; 97: 252-60.

Paper III

Osima M, Borgen TT, Lukic M, Grimnes G, Joakimsen RM, Eriksen EF, Bjørnerem Å. Serum parathyroid hormone is associated with increased cortical porosity of the inner transitional zone at the proximal femur in postmenopausal women: The Tromsø Study. Osteoporos Int. 2017; Nov 14. Doi: 10.1007/s00198-017-4298-3. [Epub ahead of print]

Abbreviations

$1.25(OH)_2D$	1.25-dihydroxyvitamin D
25(OH)D	25-hydroxyvitamin D
aBMD	Areal bone mineral density
AGEs	Advanced glycation end products
ANOVA	Analysis of variance
BMA	Bone marrow adiposity
BMD	Bone mineral density
BMI	Body mass index
BMU	Bone multicellular unit
BTM	Bone turnover markers
BV/TV	Bone volume as a proportion of the total volume
CC	Compact cortex
CI	Confidence interval
CKD	Chronic kidney disease
CSA	Cross-sectional area
CT	Computed tomography
CTX	C-terminal telopeptide of type I collagen
CV	Coefficient of variation
DXA	Dual-energy x-ray absorptiometry
eGFR	estimated glomerular filtration rate
FN	Femoral neck
FRAX	Fracture risk assessment tool
HR-pQCT	High resolution peripheral quantitative computed tomography
HRT	Hormone replacement therapy
IGF-1	Insulin-like growth factor 1
ITZ	Inner transitional zone
LOF	Level of fullness
MAS	Medullary adiposity score
MAT	Marrow adipose tissue
NOK	Norwegian krone
OR	Odds ratio
OTZ	Outer transitional zone
PTH	Parathyroid hormone
PINP	N-terminal propeptide of type I procollagen
PPARs	Peroxisome proliferator activator receptors
ROI	Region of interest
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 of fragility fractures

Bone fragility is a major health challenge globally and, clinically manifests through its association with fragility fractures, resulting in increased morbidity and mortality (1-3). Fracture rates differ by ethnicity and geographic region (4). This variation is best documented for hip fractures, with Norway and other Scandinavian countries having the highest annual age-standardized hip fracture incidence in the world (4, 5). Low-risk regions include Africa, Latin America, and Saudi Arabia, leading to the perception that countries furthest from the equator have the highest hip fracture incidence (1). Exceptions to this are Middle Eastern countries, like Oman, Kuwait and Iran. (1, 4).

Hip fracture is the most serious type of fracture due to increased mortality, morbidity, and cost to the society (6, 7). The rates of hip fracture increase with advancing age, particularly in individuals over 70 years (8). The number of elderly individuals will increase globally due to increased life expectancy, and the number of individuals older than 70 years in Norway might double in the next 30 years (1, 9). The worldwide incidence of hip fracture is estimated to increase from 1.66 million in 1990 to 6.26 million by 2050 (10). In Norway, more than 9000 individuals suffer hip fracture every year (5, 11). If rates of hip fracture in Norway remain constant, the burden is expected to double in the next 25 years (12). A diagnosis of hip fracture is one of the most expensive diagnoses, with a total costs of approximately 500000 NOK the first year after a fracture, in Norway (13). A hip fracture leads to loss of healthy life-years, reduced quality of life, and long-term nursing care (7, 14, 15), resulting in a burden to the patients and society.

Of 3.5 million fragility fractures in the European Union in 2010, 17% were hip fractures, 16% were forearm fractures, 15% were vertebral fractures, and 51% were other fractures (7). Forearm fractures predict hip fractures, and this type of fracture may therefore be a first sign of bone fragility (16). The term “fragility fracture” is often used synonymously with the term “osteoporotic fracture” or “low-energy fracture,” which is defined as a fracture that occurs with the trauma equivalent to that generated by a fall from standing height or lower (17). Individuals

who have fragile bone, or “osteoporosis”, have increased risk for any type of fracture. Fractures of the hip, wrist, vertebra and humerus are the most common types of fragility fracture.

1.2 Definition of osteoporosis

The definition of osteoporosis is “a systemic skeletal disease characterized by low bone mass and micro-architectural deterioration of bone tissue, with a consequent increase in bone fragility and susceptibility to fracture” (18). The World Health Organization`s (WHO`s) operational definition of osteoporosis is based on a measurement of bone mineral density (BMD) using dual-energy X-ray absorptiometry (DXA), and defined as a BMD 2.5 standard deviations (SD) or more below the average value for premenopausal women (T-score $\leq$ -2.5). Osteopenia is defined as a value of BMD more than 1 SD below the young adult mean, but less than 2.5 SD below this value, whereas normal bone mass is defined as a value of BMD above 1 SD of the young adult reference mean (19). Severe osteoporosis denotes osteoporosis in the presence of one or more fragility fractures. The same absolute value for BMD that is used for women can be used for men (20), and different sites can be used for measurement. The most used sites are the total hip, femoral neck (FN), or lumbar spine.

The operational definition of osteoporosis has evolved into a clinical diagnostic definition, with low BMD identifying subjects at greatest individual risk for fracture (21). BMD is a strong predictor of fracture, with 1 SD decline in BMD associated with two to three fold increase in age-adjusted hip fracture risk (22-24). Still, there is a challenge in identifying subjects with fracture risk. Most fragility fractures occur in subjects with osteopenia and even normal BMD, since this proportion of the population numerically is bigger (Fig. 1) (25-27). BMD is not the only factor determining bone strength (28-30) and fracture risk, it is therefore important to consider other risk factors for fracture.

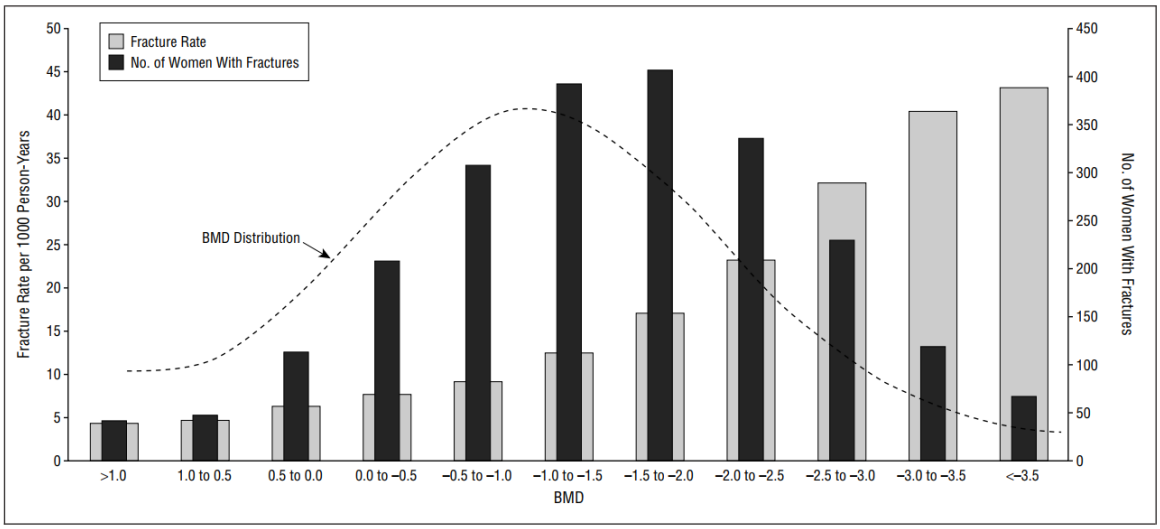


Fig. 1: *Bone mineral density (BMD), osteoporotic fracture rate, and number of women with fractures. Reprinted from Archives of Internal Medicine, Siris et al. Copyright © 2004 with permission from the JAMA network (26)*

1.3 Bone architecture, content and function

1.3.1 The skeleton and the composition of bone

The skeleton provides structural support, protects internal organs and enables mobility (31). It consists of the axial skeleton (vertebral column and thorax) and appendicular skeleton (upper and lower limbs, shoulder, and pelvic girdles). The adult human skeleton contains more than 200 bones. The skeleton must meet contradictory needs: lightness to enable movement, and strength to avoid breaking under loading and trauma (32). Bone mass is important for bone strength, but so is the structure of the bone, shape, size and the three-dimensional architecture. Important for strength is also material properties, as micro-damage, collagen, and matrix mineralization (Fig. 2) (28-30, 33, 34).

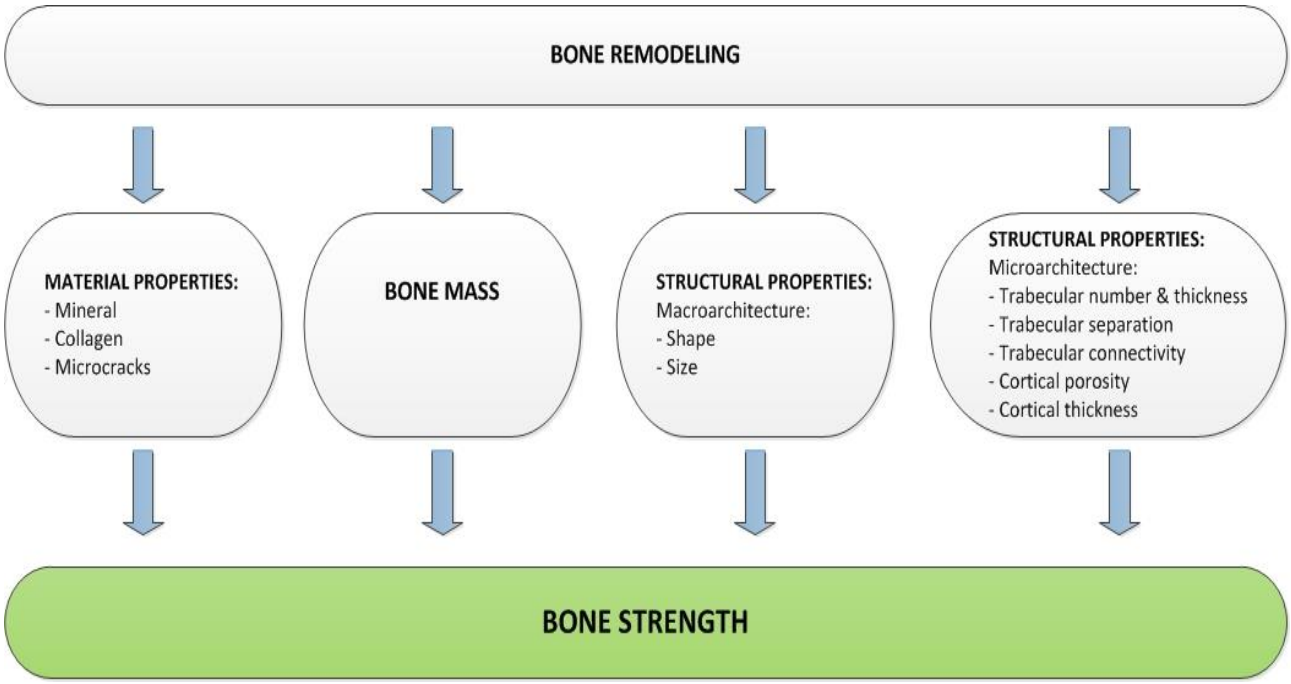


Fig. 2: *Determinants of bone strength.*

Bone is a living connective tissue and a reservoir for minerals such as phosphorus and calcium. As much as 99% of calcium in the human body is concentrated in the mineral phase of bone as hydroxyapatite crystal [$Ca_{10}(PO4)_6(OH)_2$], with the remaining 1% present in blood, extracellular fluid, and soft tissues. The extracellular matrix of bone consists of collagen (20-25%), minerals (60%), water (5-10%), noncollagenous proteins, and lipids, which are components for metabolic and mechanical functions (35). The collagen has a triple-helix structure that is organized into ropes (fibrils), and this gives the bone tissue its tensile strength, elasticity and flexibility (36). Hydroxyapatite plays an important role in the mechanical weight-bearing properties of the bone, and is a source of calcium (35). These crystals confer compressive strength and rigidity (37).

Bone matrix is a composite material that is constantly regenerated throughout life as a consequence of bone turnover (remodeling), and 5-10% of the bone tissue is replaced by new bone every year (38). Bone tissue consists of three types of bone cells: osteoblasts, osteocytes, and osteoclasts (35). Osteoblasts develop from bone marrow precursor cells: they produce the bone matrix, and promote osteoclast differentiation through secretion of cytokines (35). Osteoblasts differentiate into osteocytes, which become embedded in the bone matrix (35). Osteocytes communicate with other cells through an extensive network of canaliculi (36),

detect mechanical strain, and coordinate the bone remodeling process. Bone lining cells are former osteoblasts that cover the bone surfaces (36). Osteoclasts are large multinucleated cells derived from a monocyte stem-cell lineage. They resorb the bone matrix on bone surfaces during the initial stage of the bone remodeling process (34). All cells communicate and respond to one another through signaling molecules or direct cell contact.

1.3.2 Architecture of bone: cortical and trabecular bone

The outer part of bones, cortical bone (compact bone), accounts for 80% of the bone mass of the adult human skeleton, while 20% is trabecular bone (cancellous or spongy bone) (Fig. 3) (31, 36).

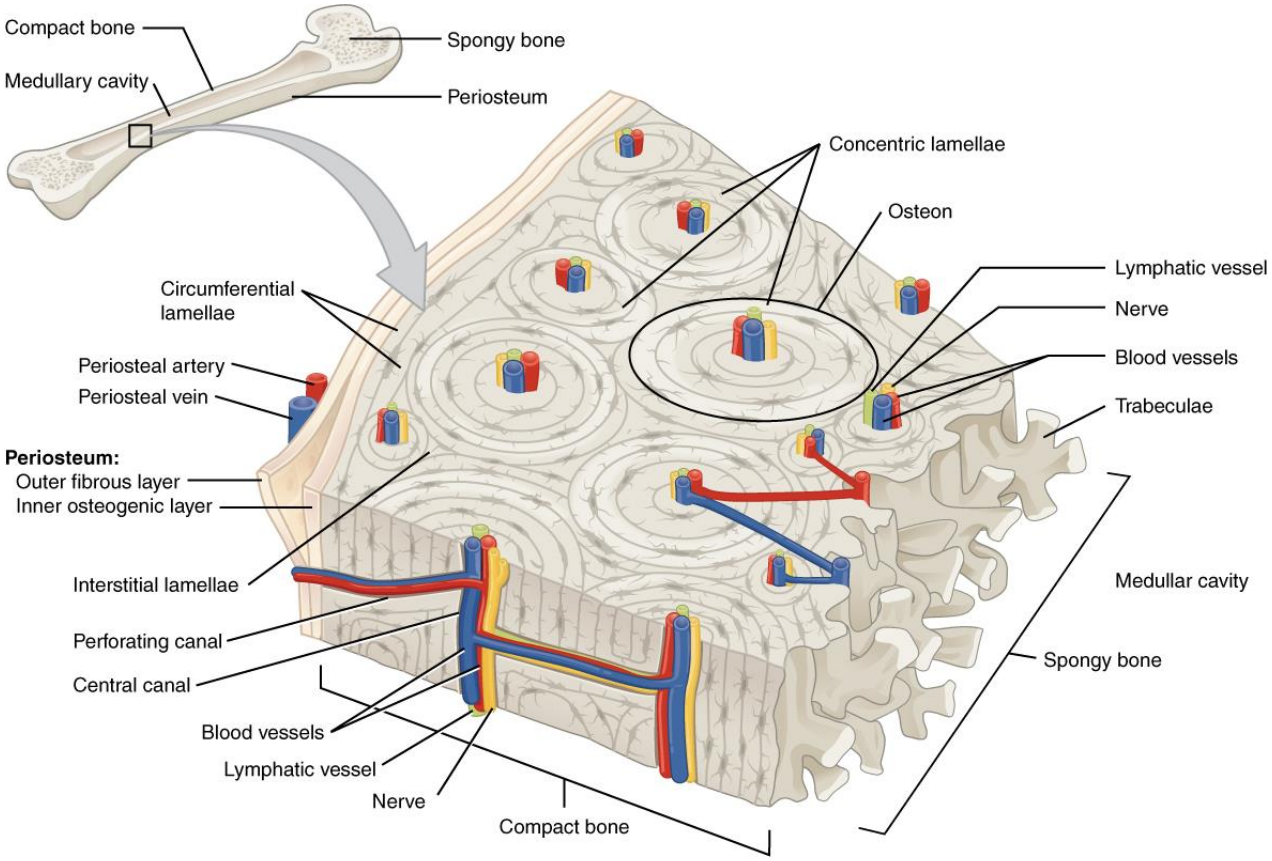


Fig. 3: *Components of bone.*
Cortical (compact) and trabecular (spongy) bone. Central canal = Haversian canal. Perforating canal = Volkmann canal. By OpenStax Anatomy and Physiology: https://cnx.org/contents/FPtK1zmh@8.25:fEI3C8Ot@10/Preface, CC BY 4.0.

Cortical bone is more dense than trabecular bone: still, it is not completely compact because it contains void or pores (39). The pores are formed mainly by the canals traversing the cortical bone (Figs. 3-5), seen as porosity in cross section (Figs. 4-5). Cortical porosity is the proportion of emptiness (void volume) of the cortical bone volume (40). In mature bone, or lamellar bone, osteocytes reside in small ellipsoidal holes called lacunae (34). These lacunae are located between the various lamellae. Dendritic processes from osteocytes extend through the canaliculi to meet with the surrounding cells. Lamellar bone is arranged in Haversian systems, cylindrical structures also called secondary osteons. The diameter of the Haversian system is approximately around 200 μm, and the length is 1–3 mm. An osteon consists of tightly packed lamellae (10–30 concentric rings), surrounding a central cavity, the Haversian canal, where blood vessels and nerves run through. Transverse vessels running perpendicular to the long axis of the cortex, are located in Volkmann canals (39) (Figs. 3 & 5).

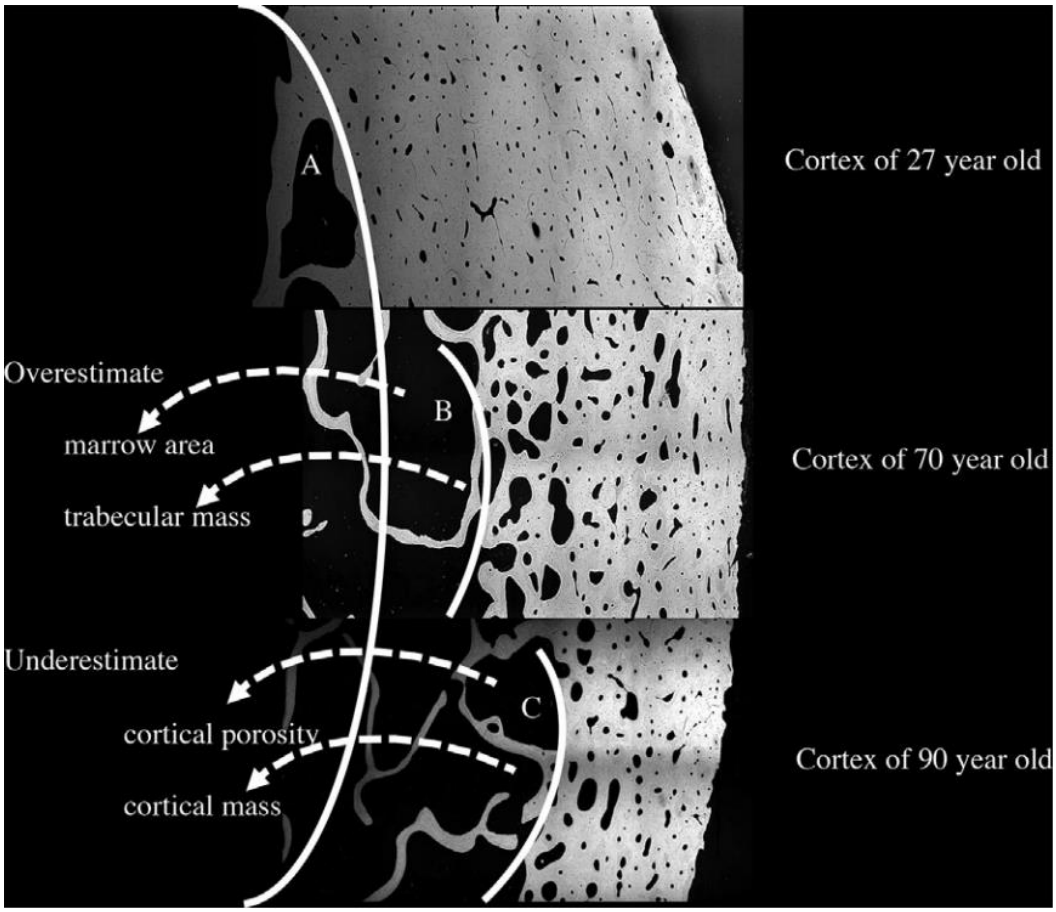


Fig. 4: *Intracortical and endocortical remodeling erode the cortex.*
The endocortical surface (white line A of a specimen from a 27-year-old) denotes the true medullary cavity/cortical interface achieved at completion of growth. If the surface of the thinned but still compact appearing cortex (white line B in a 70-year-old or C in a 90-year-old) is erroneously described as the endocortical surface, several errors occur by incorrectly apportioning in the cortical fragments and porosity that created them to the seemingly expanded medullary canal. Reprinted from JBMR, Zebaze et al., Copyright © 2015 with permission from Wiley Online Library (41).

The interface between the bone matrix and fluid-filled void, the endosteal surface, consists of three components: i) the intracortical surfaces formed by the lining of the Haversian and Volkmann canals, ii) the endocortical surfaces between the cortex and medullary canal, and iii) the trabecular surfaces on all sides of the trabecular plates (Figs. 3-5) (37, 39). Trabecular bone is a porous cancellous network of plates and rods, comprising of 10-30% mineralized bone matrix and 70-90% void volume, and the trabecular thickness is 50-300 µm (37, 42). This porous structure achieves lightness and flexibility and can absorb more energy than cortical bone by deforming more before cracking. Trabecular bone has a higher surface area/bone matrix volume ratio, than cortical bone (37). The transitional zone (TZ), or cortico-trabecular junctional zone, is the inner part of the cortex, adjacent to the medullary cavity (37, 39, 40). The trabecular bone is located in the medullary cavity of vertebral bodies, ends of long bones, and inner part of flat bones. The cortical bone is located in the shafts of the long bones and on surfaces of other bones. The femoral neck has 50% cortical and 50% trabecular bone, for comparison, the vertebral bodies consist of 25% cortical and 75% trabecular bone (36).

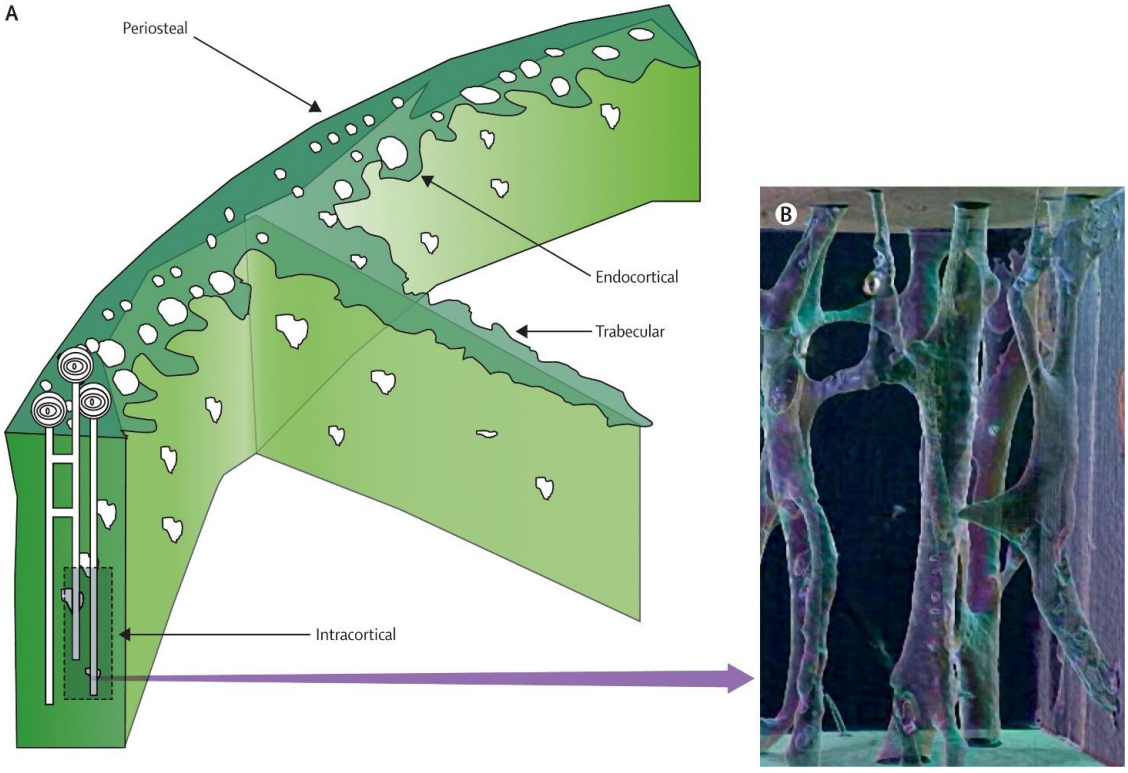


Fig. 5: *Structure of bone.*
(A) Cortical and trabecular bone, the periosteal (external) surface, and the three (endocortical, trabecular, intracortical) contiguous components of the endosteal (internal) surface on which matrix remodeling is initiated. (B) The intracortical surface formed by the lining of myriads of Haversian and Volkmann canals traversing the cortex. These canals are seen as pores in cross-section. Reprinted from The Lancet, Zebaze et al. Copyright © 2010 with permission from Elsevier Ltd (39).

1.3.3 Medullary cavity and bone marrow

In the medullary cavity (Fig. 3), there are spaces between the trabeculae that are filled with bone marrow. The bone marrow contains hematopoietic, fat, and stroma cells (43). At birth, bone marrow consists mainly of hematopoietic cells, but it is replaced with marrow adipose tissue (MAT) during skeletal growth and with advancing age (44, 45). Hematopoietic cells are erythrocytes, neutrophils, lymphocytes, and platelets, and hematopoietic stem cells (HSCs) that differentiate to myeloid, lymphoid, and erythroid lineages. The medullary cavity is responsible for hematopoiesis (46), and the hematopoietic stem cells (HSCs) develop into mature blood cells (47). In addition, multipotent mesenchymal stem cells (MSCs) can differentiate to mature adipocytes or osteoblasts (Fig. 6) (48). The bone marrow is known as red marrow when it consists mainly of hematopoietic cells, and yellow marrow when it mainly consists of adipocytes (49). The medullary cavity, with its bone marrow, is a functional unit integrating endocrine, autocrine, and paracrine signals for body homeostasis (43).

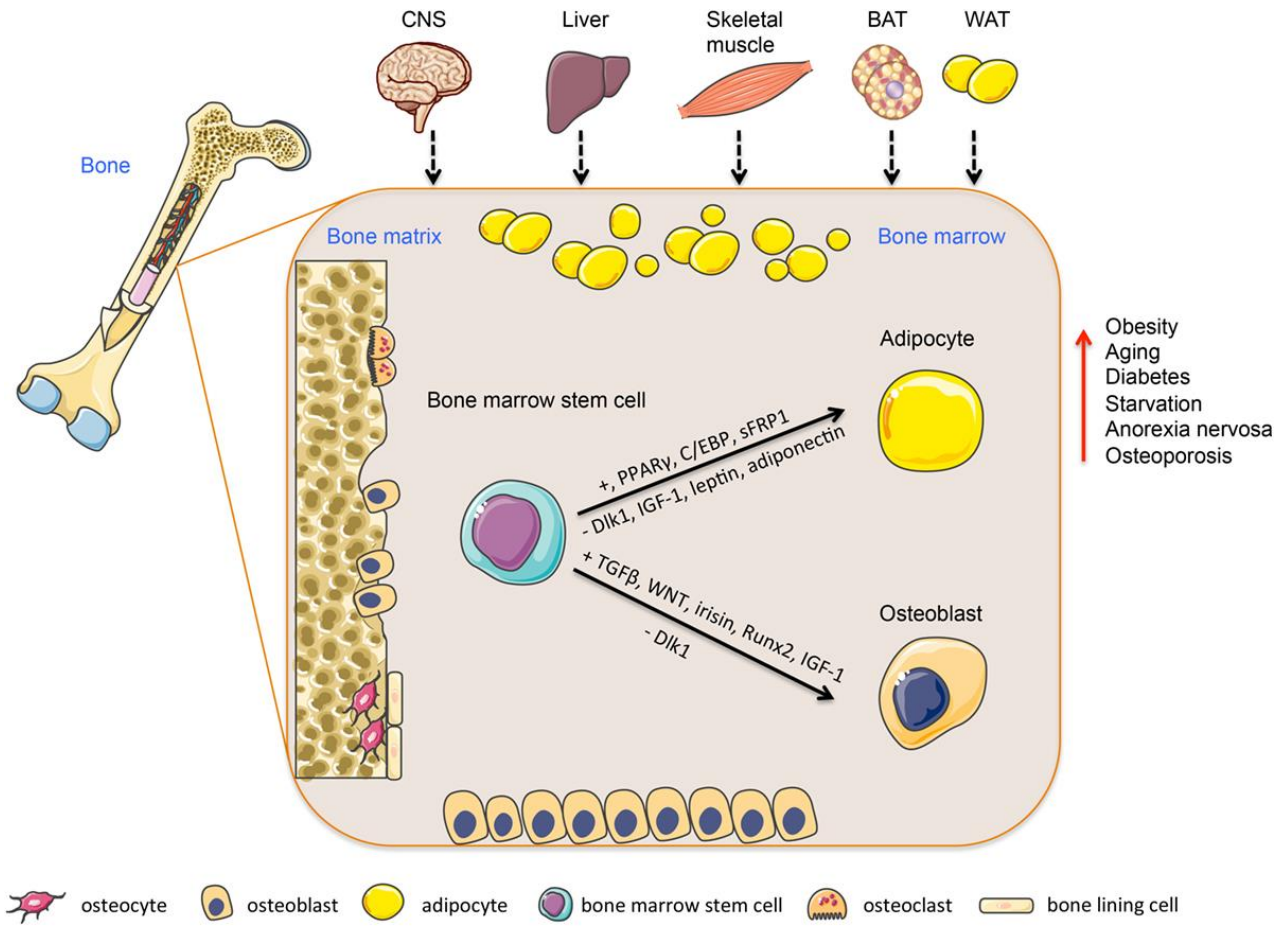


Fig. 6: *Regulation of bone marrow stem cells differentiation into adipocytes or osteoblasts. Abbreviations: CNS, central nervous system; BAT, brown adipose tissue; WAT, white adipose tissue. Reprinted from Frontiers in Endocrinology, Tencerova and Kassem, Copyright © 2016 with Open Access (48).*

1.3.4 Bone modeling and remodeling

Bone turnover or remodeling maintains bone architecture and strength by breaking down and removing old or damaged bone and replacing this with new bone (32). Remodeling happens on bone surfaces when osteoclasts remove bone tissue and cellular debris, followed by osteoblasts, forming new replacement tissue within the same location (Fig. 7) (34). The skeleton is remodeled continuously through the remodeling process occurring within basic multicellular units (BMU) formed by osteoclasts and osteoblasts (34, 50). Bone is renewed mainly on the three components of the endosteal surfaces: the endocortical, intracortical, and trabecular surfaces, and, to a small extent, on the periosteal envelope (Fig. 5) (41). Bone remodeling maintains the structure of the bone, and assists in mineral homeostasis. In healthy bone, there is a balance between expected accumulation of damage due to fatigue and daily loading, and repair of bone (30, 51). High remodeling may result in negative bone balance, low mineralization, bone loss, and increased risk for fracture (32). Low remodeling can result in accumulation of microdamage without proper renewal of old damaged bone, resulting in increased mineralization and brittleness of the bone tissue, which also may increase the risk for fracture (33). Bone remodeling and renewal of bone are therefore not necessarily making harm.

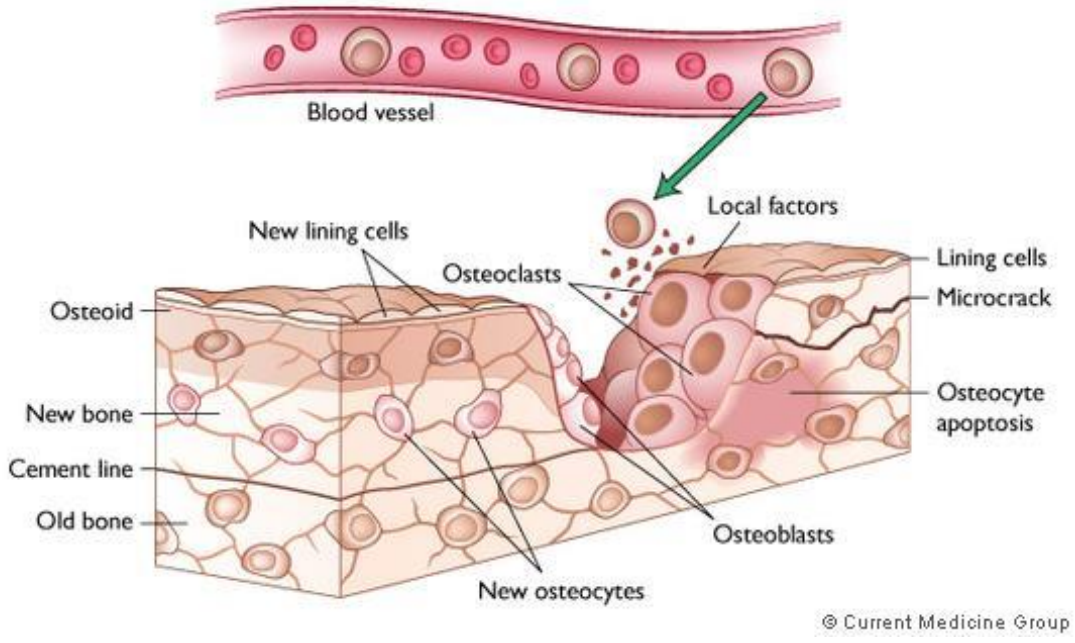


Fig. 7: *Bone remodeling in a microcrack.*
Following apoptosis of osteocytes, lining cells and osteocytes release factors attracting cells from blood and marrow. Osteoclastogenesis occur, osteoclasts resorb matrix. Osteoblasts then deposits new lamellar bone. Some osteoblasts die, some are trapped in the matrix become to become osteocytes, and other form new lining cells. Modified by Current Medicine Group Ltd, 2009 (52), from NEJM, Seeman and Delmas (34), Copyright © 2006. Reproduced with permission from Massachusetts Medical Society and Springer.

In young age, during growth, resorption is essential for the excavation of the marrow cavity, as well as the fashioning of cortical and trabecular bone (34). Since bone is broken down and replaced to the same extent, there is no net loss of bone within each of the BMU (34). Bone modeling and remodeling during growth establish peak skeletal strength. In young adults, with balanced remodeling, resorption removes damaged bone, whereas the formation phase restores the structure, a cycle resulting in restitution of bone (34). A negative balance in the BMU in aging, especially in women after menopause, causes bone loss, of cortical and trabecular bone, and reduced strength of bone (34). A smaller volume of bone is formed than removed, which results in bone loss (38). In adulthood, initially, there is relatively more trabecular than cortical loss of bone (32). Trabecular bone has more surface per unit of bone volume accessible for bone remodeling than cortical bone. In cortical bone, remodeling happens on the intracortical surfaces formed by the lining of the Haversian canals, enlarging them, which is resulting in increased porosity and on the endocortical surfaces resulting in thinning of the cortices. The remodeling on trabecular surfaces results in thinning of trabeculae that become disconnected and lost (32).

Bone turnover markers (BTMs) are measured in the serum. There are two groups of markers: those of bone formation, e.g., procollagen type I N-terminal propeptide (PINP), and markers of bone resorption, e.g., C-terminal cross-linking telopeptide of type I collagen (CTX) (53). Both PINP and CTX are bone specific, and primarily originate from bone. PINP increases rapidly during bone formation-stimulating therapy. CTX is a product of the breakdown of type I bone collagen, and rapidly decreases with antiresorptive treatment. CTX exhibits a strong circadian rhythm, so blood samples for measurement must be collected in the morning, in a fasting state (54). PINP on the other hand displays less diurnal variation and less sensitivity to food intake. Samples of CTX have to be shipped in a frozen state, while PINP is stable for weeks at room temperature. Elevated serum levels of BTMs are associated with increased risk of nonvertebral and vertebral fractures (55, 56).

Estrogen is important for bone health, during growth and aging, and low levels of estrogen is particularly involved in the pathogenesis of bone fragility after menopause (32). Low levels of estrogen increase bone remodeling and the lifespan of osteoclasts, and decreases the life span

of osteoblasts. Thus, more bone is resorbed in each of the higher numbers of BMU, and less bone is formed in postmenopausal women (32). In addition, parathyroid hormone (PTH) and 1.25 dihydroxyvitamin D_3 ($1.25(OH)_2D_3$) regulates osteoclastogenesis (57).

1.4 Risk factors for fracture and risk assessment

Knowledge of risk factors for fracture is important, to better identify subjects at high risk, and to target treatment more effectively (58). Fracture risk is associated with several factors.

1.4.1 Bone mineral density

Fracture rates increase with lower BMD, with approximately a doubling of risk for every SD decline in BMD (59). Therefore, BMD is a strong predictor for fracture (21, 22, 24). The measurement of BMD at any site predicts fracture at any site, and BMD measured at the hip is the best predictor of hip fracture. BMD at any site predicts fracture best at the same site as the BMD measurement (23, 59).

1.4.2 Age, gender, genetics and ethnicity

Age is a well-known risk factor for fragility fracture (20, 60). Most hips fractures occur after the age of 70 years (61), while distal radius fractures tend to occur in women older than 45 years (60, 62). Women fracture more than men (60, 61). Having a parent with a previous hip fracture increases the risk for a fragility fracture (20, 63). Genetic factors are important (64-66), with some genes associated with fracture (67, 68). Being a female and Caucasian are risk factors for any type of fracture (69).

1.4.3 Falls and previous fracture

Most fragility fractures occur due to falls (70), and falls predict new falls. This is particularly a challenge in the oldest and most fragile patients (71-73). Moreover, another important risk factor is a prior fragility fracture after the age of 50 years, which double the risk for a new fracture (74).

1.4.4 Anthropometry and lifestyle

Increasing height is associated with fragility fracture (75-78). The association between BMI and fracture risk is complex and affected by the interaction between BMD and BMI (79), and a consensus regarding this issue has not yet been reached (80). Low BMI has been reflected upon as a risk factor, and obesity has been considered protective for fracture due to load on weight-bearing bones, resulting in a positive effect on BMD (81-83). In recent years, studies have reported that obese adults are more prone to fracture of the ankle and upper leg (84) and humerus (83). A meta-analysis reported that high BMI remained a risk factor for upper arm fracture (humerus and elbow) when adjusted for BMD, and was further a risk factor for all osteoporotic fractures. Osteoporotic fractures in this analysis were fractures of the spine, coccyx, ribs, pelvis, humerus, forearm, elbow, hip, other femoral, tibia and fibula, clavicle, scapula and sternum (79). Physical activity is good for bone health, since weight-bearing activities increase BMD (85), and activity protects against hip fracture (86, 87) and other fractures (88). Current smoking increases the risk for fracture (89), so does alcohol consumption of three or more units daily (90). Nutrition is important, especially insufficiency of vitamin D and proteins might affect bone (91, 92).

1.4.5 Co-morbidities and medication

Chronic diseases are causing secondary osteoporosis and increase the risk for fractures (90), e.g., rheumatoid arthritis (90) and inflammatory bowel disease, partly because of glucocorticoid use (93, 94). Also important are organ transplantation (95-97) and immobilization over a long period (98, 99). Early menopause and endocrine conditions, such as untreated hypogonadism in women and men (oophorectomy or orchiectomy), hypopituitarism, anorexia nervosa (100-106), hypothyroidism or hyperthyroidism (107, 108), secondary hyperparathyroidism, chronic kidney disease (CKD) (109), and type 1 and 2 diabetes mellitus (110-114) play a significant role.

1.4.6 Diabetes mellitus and bone turnover markers

Both type 1 (T1DM) and type 2 diabetes mellitus (T2DM) are risk factors for fragility fracture (110-112, 115). They both manifest as low bone turnover conditions (116-118), which are normally associated with reduced fracture rate. Increased cortical porosity has been suggested as a reason for the increased risk for fragility fracture in patients with T2DM (119-123), even

though diabetes is a condition with low bone turnover, and high bone turnover is associated with fragility fracture (55, 56). Decreased cortical porosity in subjects with T2DM is also reported (124), so is increased bone marrow adiposity (BMA) in individuals with both T1DM and T2DM (46, 118). Therefore, the pathophysiology is still controversial.

In patients with T1DM, several reasons for increased fracture risk have been suggested. Decreased levels of amylin and insulin in T1DM, resulting in less anabolic effect on bone, might lead to impaired bone function through a decrease in insulin-like growth factor 1 (IGF-1) concentration (125). Poor glycemic control has a negative impact on bone mass, and osteoblast dysfunction or altered activity have been reported from *in vitro* studies after long-term exposure to high glucose (125, 126). Advanced glycation end products (AGEs) because of high levels of glucose, are suggested to cause brittleness of collagen, and thereby microdamage in the bone matrix (127, 128). If T1DM manifests itself before skeletal growth is complete, it may also result in decreased peak bone mass (125). Subjects with T2DM normally have higher BMI and areal BMD (aBMD) than the general population, and, despite this, they have increased fracture risk (113, 114). Several mechanisms of fracture risk increase in subjects with T1DM also apply to subjects with T2DM, as the latter individuals have poor glycemic control over time, resulting in accumulation of AGEs, and changes in osteoblast receptor signaling, or even apoptosis of osteoblasts (126-128). For advanced stages of diabetes mellitus, complications such as retinopathy and neuropathy, leading to poor balance and falls, are suggested to result in increased fracture risk (111), but this is still debated (114).

1.4.7 Parathyroid hormone, vitamin D, and calcium

The parathyroid glands secrete parathyroid hormone (PTH). PTH synthesis and release is regulated by serum calcium. The term vitamin D is utilized for both vitamin D_2 (ergocalciferol) and vitamin D_3 (cholecalciferol) (129). Vitamin D3 is obtained from fatty fish, and vitamin D enriched food (130), and it is synthesized from 7-dehydrocholesterol in skin exposed to ultraviolet radiation from the sun (131). It is further transported to the liver, and hydroxylated to 25-hydroxyvitmanin D (25(OH)D), a stable metabolite measured in the serum (129).

Hyperparathyroidism, both primary and secondary, due to chronic kidney disease, low calcium intake, or 25(OH)D deficiency, are associated with increased risk of fracture (90, 132, 133). Fracture susceptibility in individuals with low 25(OH)D are also reported following reduced muscle strength, with associated risk of falls (134, 135) PTH is secreted from cells in the parathyroid gland, in order to maintain normocalcemia, and it responds to small decrements in serum calcium. A decrease in serum calcium leads to secretion of PTH from the parathyreoidea, which stimulates renal hydroxylation of 25(OH)D to $1.25(OH)_2D$. $1.25(OH)_2D$ regulates calcium and phosphate homeostasis via bone, kidney and gut (136). It increases the bone resorption releasing calcium from the skeleton, it reduces urinary calcium excretion, increases phosphate excretion, and enhances intestinal absorption (129, 130). PTH and vitamin D together regulate the calcium metabolism, in balance with calcitonin, which stimulate deposition of calcium in the bone. Both $1.25(OH)_2D$ and serum calcium contribute to negative feedback inhibition of secretion of PTH (129).

$1.25(OH)_2D$ mediates its effect directly on bone cells, and indirectly by stimulating intestinal absorption of calcium and phosphorus and increases the efficiency of the absorption (129). In the kidney, $1.25(OH)_2D$ enhance the actions of PTH on calcium transport in the distal tubule. An important target for $1.25(OH)_2D$ is the parathyroid glands, where $1.25(OH)_2D$ inhibits synthesis and secretion of PTH, and also prevents proliferation of parathyroid-producing cells (129).

The target treatment threshold levels of 25(OH)D, as well as the most favorable level required for bone health has been debated (131, 137, 138). A level < 50 nmol/L is indicated, and clinically used, as a threshold for vitamin D inadequacy, when taking BMD, bone turnover, muscle function and falls into account (139). Moderate vitamin D deficiency is defined as serum 25(OH)D of 12.5-25 nmol/L, and severe deficiency are levels under 12.5 nmol/L (131).

1.4.8 Bone marrow adiposity

BMA is inversely associated with BMD (140), and vertebral BMA is associated with compression fractures independent of vBMD (141). Marrow adipose tissue (MAT) and bone tissue reside side-by-side, and there is a common mesenchymal progenitor cell for the adipocytes and osteoblasts (Fig. 6) (142). A question has been raised regarding whether MAT is an inactive filling of the medullary cavity, when hematopoiesis is impaired or bone mass is lost (143). However, MAT is now considered as a dynamic tissue that responds to hormonal, nutritional, and environmental stimuli, expanding both in response to a diet high in fat as well as calorie restriction (43, 49, 144). Anorexia nervosa is one example of calorie restriction associated with increase in bone marrow fat, with a reduction in numbers of peripheral adipocytes, whereas an expansion of peripheral adipose tissue might also result in gain in MAT (145, 146).

In a small study, vertebral MAT correlated with visceral adipose tissue in postmenopausal women with T2DM (147). The amount of MAT is influenced by endocrine signals in the absence of PTH signaling, excess of glucocorticoid, and estrogen withdrawal (144, 148). T1DM, anorexia nervosa, aging, and use of rosiglitazone are associated with expansion of marrow adipocytes, and excess MAT, and stromal cell fate is believed to be altered in these conditions, with a shift towards adipogenesis (142), even though the association may not be causal (143). These conditions are associated with increased bone resorption and reduced bone formation (149, 150). The microenvironment of the bone marrow also plays an important part in bone health, providing cytokines that contribute in the bone metabolism (151). Adiponectin, secreted primarily by adipocytes, regulates energy metabolism and affects insulin sensitivity (143). There is an interdependence between adipogenesis and osteogenesis (46, 151, 152), inverse relationship between osteogenic and adipogenic differentiation is partly mediated through cross-talk between pathways, activated by steroid receptors, peroxisome proliferator activator receptors (PPARs), and other paracrine and cytokine factors. PPARs are known to initiate adipogenesis in the bone marrow (Fig. 6) (151, 153).

2 Rationale and aims

Bone fragility has over the last 30 years partly transformed from being seen as an inevitable consequence of aging, to a condition that can be assessed, and for which there are more effective pharmacological therapies (1). However, despite advances in therapies, assessment of fracture risk, and diagnosis of bone fragility, few women and men with high fracture risk receive treatment, even after they develop fracture (154). For clinicians, to be able to recognize and identify subjects who are at risk for fragility fracture, and to target treatment well, it is important to constantly search for risk factors that are associated with, or ideally, predict fracture (21, 155). Several clinical risk factors for fracture are incorporated in the Fracture Risk Assessment Tool (FRAX), the most commonly used web-based tool globally, with or without BMD (1, 21, 90). The use of clinical risk factors in addition to measurement of BMD has increased the accuracy of fracture risk assessment (21). This is important, since the most common approach used to assess fracture risk, a measurement of BMD using DXA, has low sensitivity for fracture (Fig. 1) (20), and most fractures occur in women with osteopenia or normal BMD, not osteoporosis (25, 26). A thorough understanding of the pathophysiological mechanisms involved in associations of risk factors with fracture, is important.

In a sub-study of the Tromsø Study, cortical porosity was associated with nonvertebral fracture, independent of BMD and FRAX score, and BTMs were associated with higher odds for fracture (156). In the last 15 years (49, 142), BMA has also received increasing attention, in relation to bone fragility and fracture (45, 157, 158). BMA has not been known as a risk factor for fracture, but it has been argued that MAT is involved in the pathophysiology of bone fragility, and is more than passively filling up empty space (49). Moreover, BMA increases by advancing age, is inversely associated with trabecular bone volume (45, 46, 141), but no association with cortical porosity has been reported. We wanted to investigate whether there was an association between medullary adiposity score (MAS) and impaired cortical and trabecular microarchitecture, with focus on cortical porosity, and between MAS and fragility fracture, in women. Further, we wanted to explore whether a combination of MAS and cortical porosity identified more women with fracture than either trait alone. We originally planned to explore these associations using data from the Tromsø sub-study. However, since the subtrochanteric site contains little trabecular bone; the fat proportion of the medullary cavity could not be calculated in the Tromsø data. Instead, we used data from a case-control study of women with

and without fracture from Melbourne, Australia. Images were obtained at the distal tibia and distal radius using high-resolution peripheral quantitative computed tomography (HR-pQCT), to explore the associations between medullary adiposity score, cortical porosity and odds for nonvertebral fracture.

In overweight diabetic individuals, there is a clear correlation between MAT and glycemic control, the higher the HbA1c, the more MAT (147). We wanted to further explore some risk factors for fracture in relation to bone microarchitecture. T2DM is a modest risk factor for fracture, however, given the large number of individuals with this disease, fracture remains a major clinical concern in subjects with this condition (126). New technology is available for bone measurements *in vivo*, and there is increasing interest related to cortical and trabecular architecture as risk factors for fracture, and their role in the pathophysiology of bone fragility (25, 32, 159, 160). Increased cortical porosity has been invoked as a possible factor for fracture in T2DM patients (119-121). This statement is hard to explain, since T2DM is a low bone turnover condition (56, 117, 161), and we would rather expect that T2DM and low bone turnover would be associated with decreased porosity. We wanted to explore these associations in a cohort from the Tromsø Study.

The Tromsø Study has focused a lot on 25(OH)D and bone health with 2-dimentional measurements of BMD using DXA. Low serum 25(OH)D is associated with secondary hyperparathyreoidism and risk for fracture (131-133, 162, 163), but whether this is mediated via increased cortical porosity is not clear. Therefore, we wanted to explore this in the data from the Tromsø cohort.

We tested the following hypotheses in Paper I, II and III:

Higher medullary adiposity score is associated with higher cortical porosity and fewer trabeculae measured at the distal tibia and distal radius. (I)

Higher medullary adiposity score is associated with increased odds for detecting women with prevalent nonvertebral fracture. (I)

Medullary adiposity score and cortical porosity are each independently associated with nonvertebral fractures, and identifying more women with fractures than either trait alone. (I)

Postmenopausal women with type 2 diabetes mellitus have lower bone turnover markers and lower cortical porosity than those without diabetes. (II)

Higher serum levels of glucose and body mass index are associated with lower bone turnover markers, and with lower cortical porosity. (II)

Serum 25-hydroxyvitamin D and parathyroid hormone are associated with cortical porosity. (III)

Associations of 25-hydroxyvitamin D and parathyroid hormone with fracture risk are dependent on cortical porosity. (III)

3 Materials and Methods

3.1 Study population

Paper I

We recruited 84 women 40-70 years of age within 14 days of nonvertebral fracture occurrences, to minimize the likelihood of post-fracture changes in cortical porosity or medullary composition (Fig. 8) (164). These women presented to the Emergency Department (ED) at the Austin Hospital, Heidelberg, Melbourne, Australia during 2008-2012. The 84 fracture cases had fracture at the distal forearm (n = 52), upper arm (n = 5), elbow (n =5), hand (n = 2), rib (n =1), hip (n = 3), lower leg (n = 6), ankle (n = 9), and toe (n =1). After excluding 5 women receiving hormone replacement therapy (HRT), and 2 and 10 women with movement artifact during image acquisition of distal tibia and distal radius, 77 and 69 women with fracture remained with valid measurements of distal tibia and distal radius, respectively. We compared the measurements with those of healthy twins from the Twins Research Australia (n = 653) who were recruited during 2008-2012 (65, 75, 161). Among the controls, we excluded 30 women receiving HRT, 108 women < 40 years of age and 170 women with a prior fracture, 11 and 37 women with movement artifacts of distal tibia and distal radius, leaving 334 and 308 controls 40-76 years of age, with valid measurements of distal tibia and distal radius, respectively.

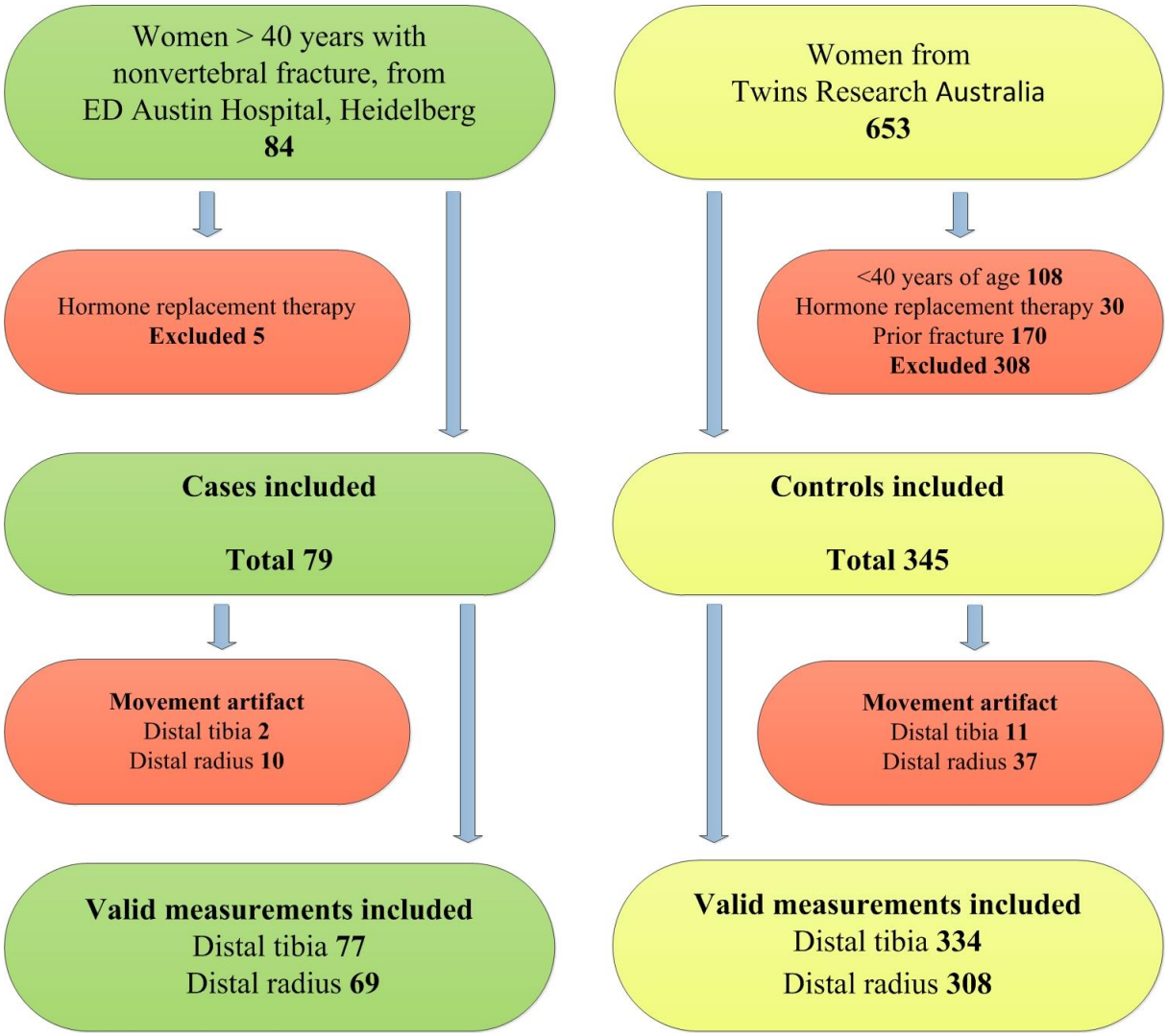


Fig. 8: *Participants in the Melbourne based case-control study in 2008-2012.*

Paper II and III

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 (165). 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 (165). During the 4th survey (Tromsø 4) 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. 9). In these participants, all nonvertebral fractures that occurred between January 1, 1994 and January 1, 2010, were registered in the University Hospital of North Norway (UNN) Tromsø X-ray archives (166, 167). There is no other radiological service or

fracture treatment service within 250 km of Tromsø. Therefore, the only undocumented fractures were those that occurred while inhabitants were traveling and for which no control radiographic examination was performed after returning home. The fracture registry includes information on the time of fracture and number and anatomical locations of all the fractures experienced by the Tromsø Study participants, and has been previously validated (166-168). 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, as a sub-study of the Tromsø study, and identified 1,250 women from the X-ray-based fracture registry who experienced at least one fracture of the hip, wrist, or proximal humerus after the age of 50 years (Fig. 9) (168). 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. 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 influence on the results. After screening, 264 fracture cases were included in the study.

Age-matched, fracture-free women who were within the same 5-year age groups were randomly selected from the Tromsø 4 cohort and 1,186 were invited to participate. After a pre-screening phone call to determine whether they were eligible and fracture-free, 260 controls were included.

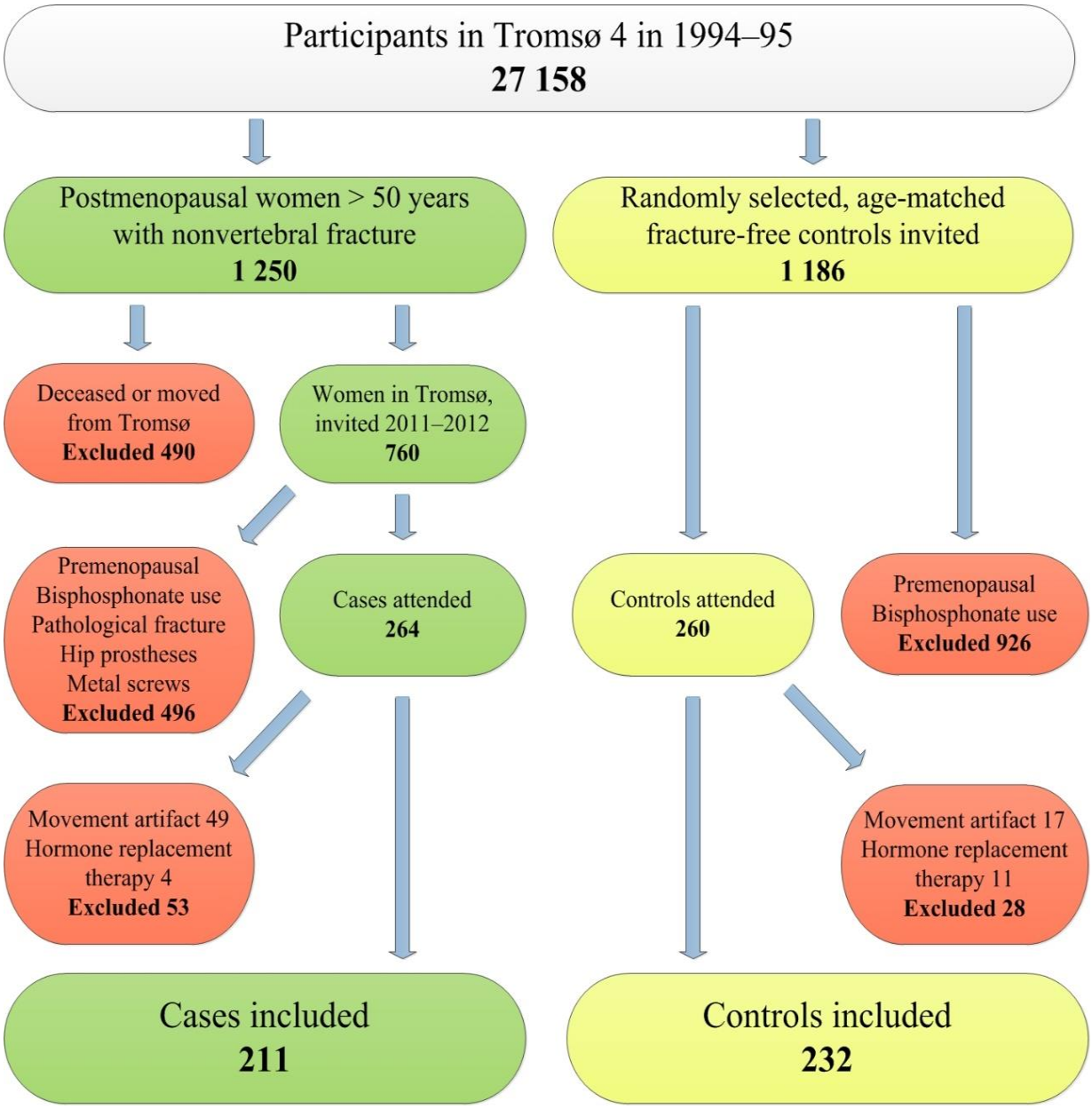


Fig. 9: *Participants in the nested case-control study based on the Tromsø 4 Study from 1994-95.*

Of these 524 participants, we excluded 15 women who were currently receiving HRT (4 cases and 11 controls) and 66 women owing to movement artifacts during CT scans (49 cases and 17 controls). Movement artifacts occur with voluntary or involuntary patient movement during image acquisition and appear as blurring, streaking, or shading on the CT image and degraded image quality. The excluded cases with movement artifacts (n = 49) were 3.2 years older than cases without movement artifacts (n = 215) (71.6 ± 1.2 vs. 68.3 ± 0.5), (p = 0.010). The excluded controls with movement artifacts (n = 17) were non-significantly 1 year older than controls without movement artifacts (n = 246) (69.2 ± 2.2 vs. 68.2 ± 0.4), (p = 0.569). Thus, 443 women

were included in the final analyses: 232 controls and 211 cases (4 hip, 181 wrist, and 26 proximal humerus fracture). The median time since their index fracture was 6.6 years (range, 1–25). All cases and controls were recruited and had their measurements obtained during November 2011-January 2013.

3.2 Ethics

Paper I: All participants provided written informed consent. The case-control study conducted in Melbourne was approved by the Austin Health Human Research Ethics Committee (H2008/03151).

Paper II and III: The sub-study of the Tromsø Study was approved by the Regional Committee for Medical and Health Research Ethics (REK Sør-Øst reference 2010/2282). Studies in Melbourne and Tromsø were conducted in accordance with the World Medical Association Declaration of Helsinki.

3.3 Data from questionnaires

Paper I: In the case-control study in Melbourne, the participants answered a questionnaire including information on their lifestyle, prior fracture, diseases, and use of medication (**Appendix A**).

Paper II and III: 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, self-perceived health, chronic diseases, use of medication and supplementation of calcium and vitamin D, and lifestyle factors such as exercise and current smoking (**Appendix B**). Hours of exercise per week were calculated as weekly exercise frequencies multiplied by hours per session. In addition, the self-reported diagnosis and duration of T2DM, and diabetic complications were confirmed based on information in medical records. None of them had T1DM.

3.4 Blood samples, anthropometry and bone mineral density measurements

Paper II and III

Fasting blood samples were collected between 8 and 10 AM and assayed 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 the UNN; for 25(OH)D (mass spectrometry with a CV of 5%); for PTH (Immulite 2000 with a CV of 7-12%); for creatinine and calcium measured photometrically with a CV of 3% and 2% at Haukeland University Hospital, Bergen; and for PINP and CTX using electrochemiluminescence immunoassays (Elecsys 1010 Analytics, Roche Diagnostics, Germany with a CV of 3–8%) at the Hormone Laboratory of Oslo University Hospital Aker. Homeostatic model assessment of Insulin Resistance (HOMA-IR) was calculated using the following formula: (glucose multiplied by insulin)/135 (169). Kidney function was assessed using estimated glomerular filtration rate (eGFR), which was calculated using the Chronic Kidney Disease Epidemiology Collaboration equation (CKD-EPI) (170). Corrected serum calcium was calculated as serum-calcium concentration + 0.0227 × (46 – serum-albumin concentration), with a CV of 2%.

Height, weight and bone mineral density measurements

Paper I, II and III

At both study sites (Melbourne and Tromsø), height and weight were measured while wearing light clothing and no shoes. BMI was calculated as weight/height^2 (kg/m^2). Total hip and FN aBMD were measured at the non-dominant side (DXA, GE Lunar Prodigy, Lunar Corporation, Madison, WI, USA). In women with a hip fracture on the non-dominant side, the opposite side was used. The coefficients of variation (CV) were ranging from 1.2% to 2.6% at the two study sites. The women were categorized into those with normal FN aBMD (T score > -1.0), osteopenia (T-score between -2.5 and -1.0) and osteoporosis (T-score < -2.5) using the WHO classification (19).

3.5 Quantification of bone architecture

Paper I

High-resolution peripheral quantitative computed tomography (HR-pQCT, XtremeCT, Scanco Medical AG, Brüttisellen, Switzerland, isotropic resolution of 82 μm) (Fig. 10) was used to obtain images at the nondominant distal tibia and distal radius (164, 171). Images were obtained with the X-ray source potential set to 60 kVp and a current of 900 μA. In those with fracture at the nondominant side, the opposite side was scanned. The 110 CT slices were obtained at a standardized distance of 22.5 and 9.5 mm from a reference line that was manually placed at the endplate of the distal tibia and distal radius, respectively (Fig. 11). The 49 most proximal slices in 110 slices of region of interest were chosen because the thicker cortex allows accurate assessment of cortical porosity. The most distal slides often have very thin cortices, so these voxels may contain background void, trabecular bone or medullary void. Therefore, they are not suitable for quantification of cortical porosity (40).

Fig. 10: *HR-pQCT, XtremeCT, Scanco Medical AG.*
Scanning of tibia (left) and radius (right). Reprinted with permission from Scanco Medical XtremeCT.

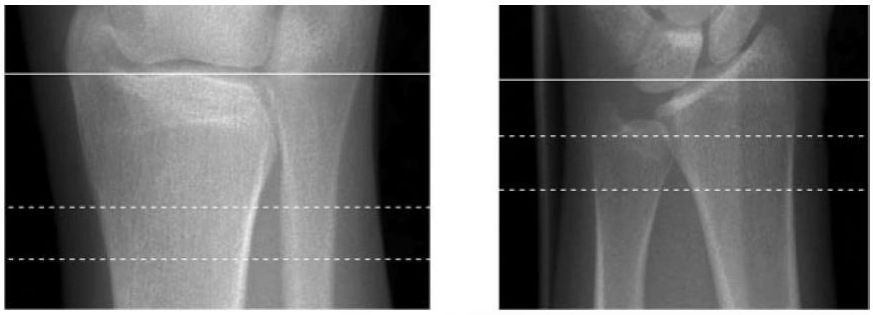


Fig. 11: *The reference line position and the measurement site:*
Images of the distal tibia (left) and distal radius (right), demonstrating the reference line position (solid line) and the measurement site (between the dotted lines). Reprinted from The Journal of Clinical Endocrinology & Metabolism, Boutroy et al., Copyright © 2005 with permission from Oxford University Press (172).

Cortical and trabecular morphology and marrow adiposity were quantified using StrAx software (StraxCorp, Melbourne, Australia), a non-threshold based method to analyze the images that automatically segmented the bone into its compartments using curve profile analysis (Fig. 12) (40).

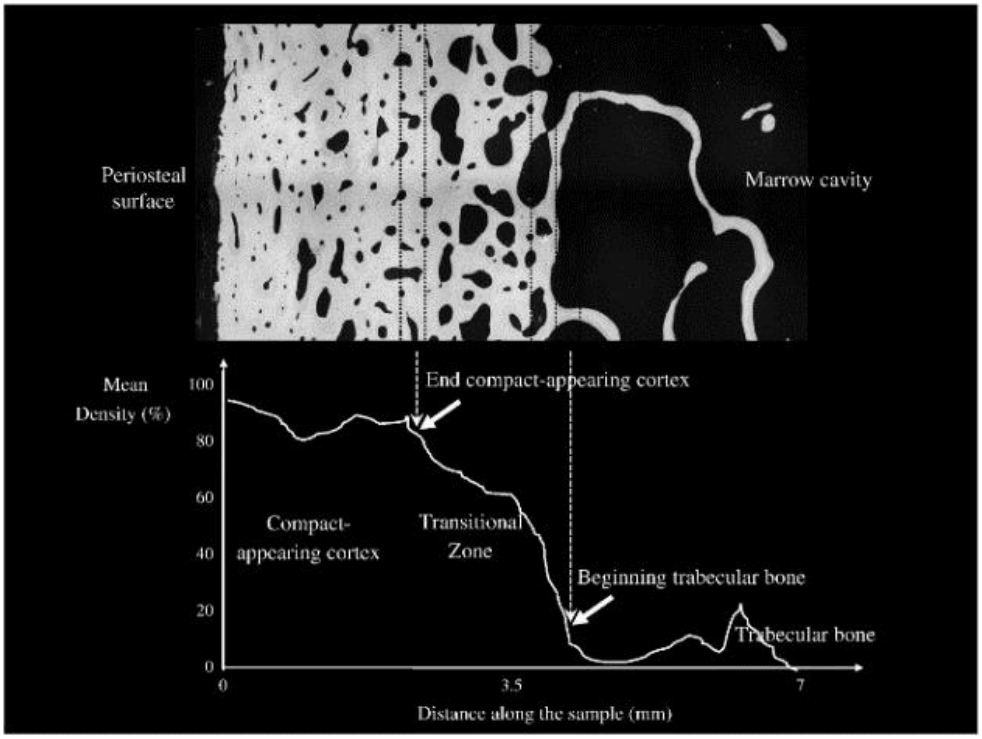


Fig. 12: *Scanning electron microscopic image of the subtrochanteric area:*
Scanning electron microscopic image of the subtrochanteric showing the compact-appearing cortex, transitional zone, and trabecular compartment. The density profile curve produced has two plateaus; one corresponding to the compact-appearing cortex and one corresponding to the trabecular compartment. Between these plateaus is a descending S shaped curve or transition. 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 (40).

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 (40). 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 transitional zone (TZ). The density profile curve expressed the mineralized bone area as the percentage of total area within each column (40). StrAx algorithm segments cortical bone into its compact cortex, outer (OTZ) and inner transitional zones (ITZ) (Fig. 12-13). The cortical porosity and cortical fragments that look like trabeculae produced by intracortical remodeling are both confined to the TZ, and not erroneously allocated to the medullary canal – a segmentation error that underestimates cortical porosity, and overestimates trabecular density (40, 41). Cortical porosity was the average void volume fraction within the compact cortex, OTZ and ITZ and total cortex (compact cortex + OTZ + ITZ) (65). Also trabecular number, thickness, separation, trabecular bone volume per tissue volume (BV/TV) were quantified.

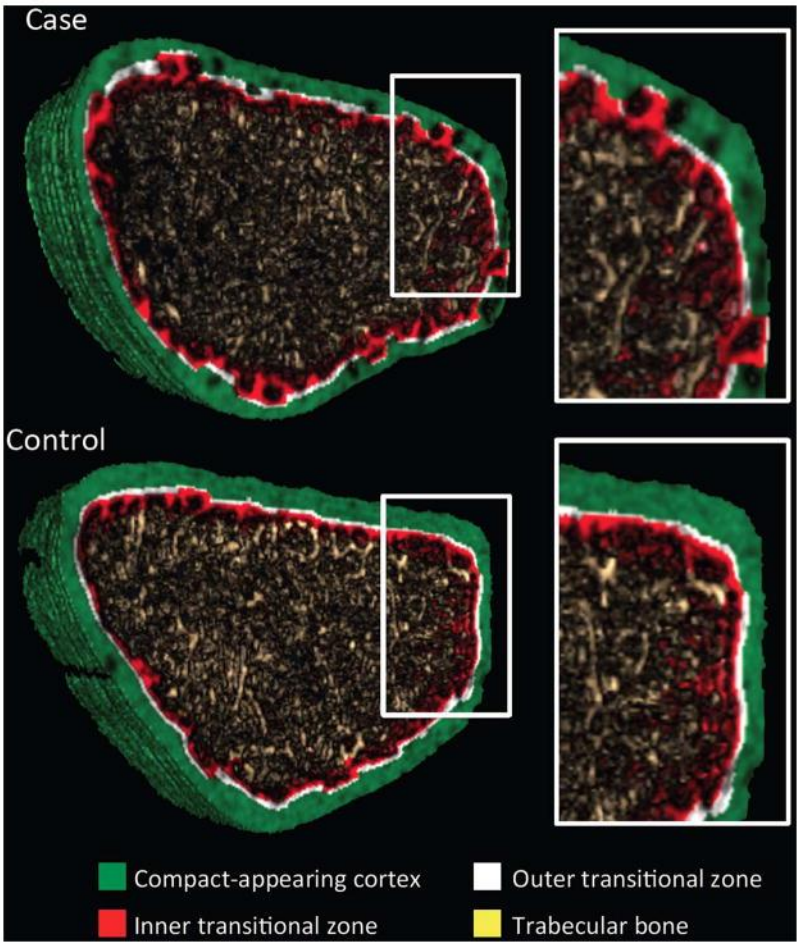


Fig. 13: *Representative segmented image obtained at the ultradistal radius:*

Representative segmented image obtained at the ultradistal radius using non-threshold-based image analysis in postmenopausal women with (case) and without (control) forearm fracture.

The full cross section and the magnified image show the presence of porosity within the compact-appearing cortex (green) and the outer (white) and inner (red) transitional zones, and loss of trabecular bone (yellow) in the case and less so in the control. Reprinted from J Bone Miner Res, Bala et al. Copyright © 2014 with permission from Wiley Online Library (25).

To quantify 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) (Fig. 14) (40). The proportion of the voxel volume occupied by mineralized bone matrix volume is its level of fullness (LOF) (along the x-axis). As previously reported, the LOF of each voxel is estimated as (LOF) % = (Ai-P)/(B-P), where Ai is the attenuation of voxel *I* (along the y-axis) (40). From the LOF, the void volume of each voxel or level of emptiness (porosity) = 100 – LOF (%) (159).

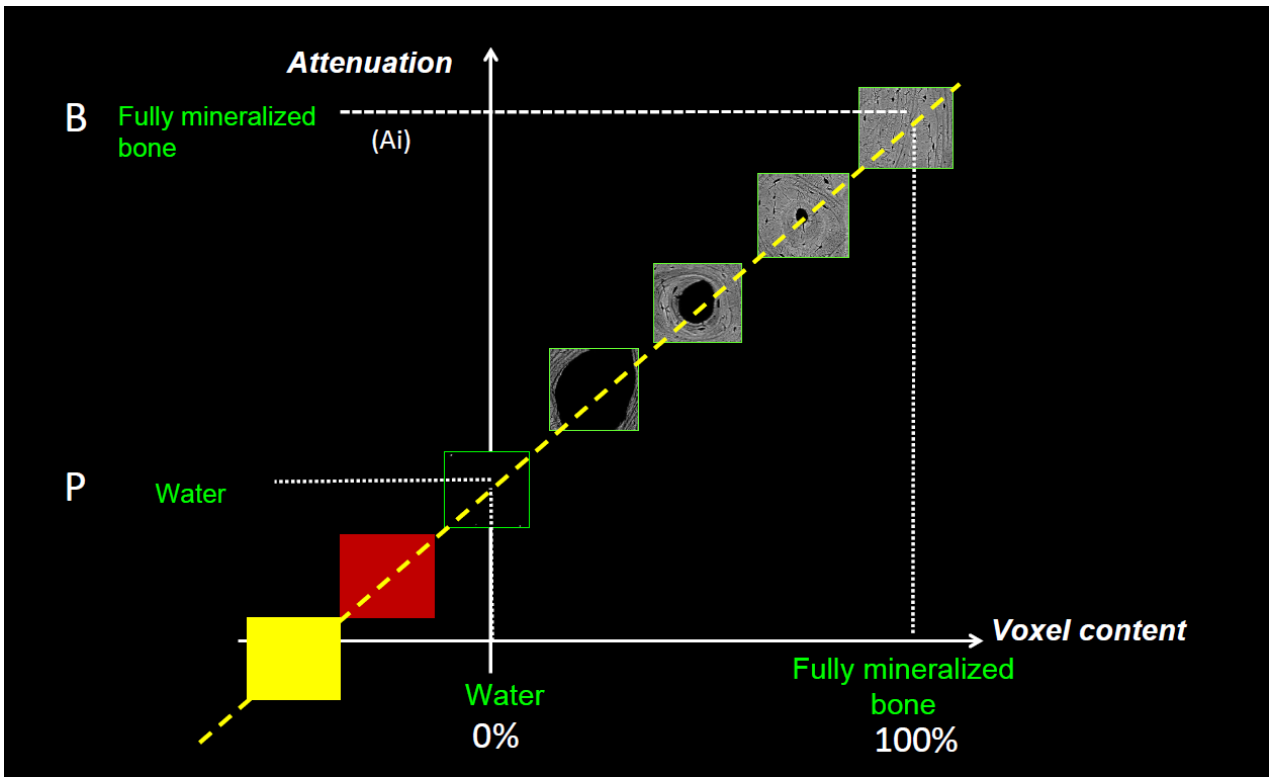


***Fig. 14**: Measurement of cortical porosity and medullary content*
To measure porosity, two referent attenuation values are required: P, the background (muscle, water etc.) and B, the fully mineralized bone matrix (1,200 mg HA/cm³). The proportion of the voxel volume occupied by mineralized bone matrix is its level of fullness (LOF) and is estimated as (LOF) %=(Ai-P)/(B-P), where Ai is the attenuation of voxel i. From LOF, the void volume of each voxel or level of emptiness (porosity)=100–LOF (%). Voxels with density below that of water are color-coded in a scale ranging from red to yellow depending on their attenuation relative to water. The lower the medullary density, the more fat cells. Reprinted and modified from Osteoporos Int, Ahmed et al. Copyright © 2015 with permission from Springer (159).

StrAx 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 StrAx 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.

This is a density-based, indirect measure of porosity, and the size and number of pores were not determined (40, 159, 173).

Medullary Adiposity score

The medullary canal contains fat cells, mineralized matrix, nonfat cells and water. Voxels containing fat cells can be identified because their photons attenuation is below that of water. We expressed the fat volume (FV) as a percentage of the medullary cavity volume (MCV) and the fat proportion (FP) $= 100 * \frac{\text{FV}}{\text{MCV}}$ (%). As age-related endocortical resorption upon the endocortical surfaces, which increases the MCV, this reduces the fat proportion but not the nonmineral apparent density produced by the fat cells, nonfat cells and water. We calculated a relative medullary density (RMD) as a percentage of fully mineralized bone matrix (1200 mg HA/cm³). As the RMD becomes more negative as fat cells increase, we subtracted this value from 100 for ease of comprehension, and RMD (%) $= 100 - [100 * \frac{\text{Mean Medullary Density}}{1200}]$

(Fig. 15). The Medullary Adiposity Score (MAS) was calculated as a function of the fat proportion and the relative medullary density, MAS $= \frac{\text{FP}*\text{RMD}}{100}$. This capture the two determinants of medullary adiposity, the fat proportion and the medullary density in a single combined variable.

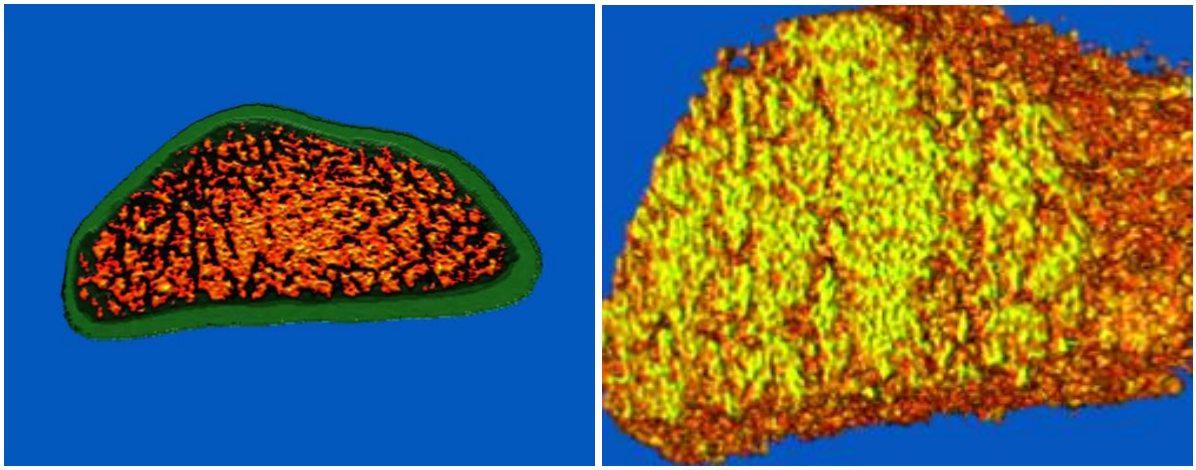


Fig. 15: *Separation of cortical bone from the medullary cavity at the distal radius*
Separation of cortical bone (green) from the medullary cavity at the distal radius (left panel). The content of the medullary cavity are color-coded in a scale ranging from red to yellow depending on their attenuation relative to water (right panel). Modified from Osima et al., submitted JBMR (164).

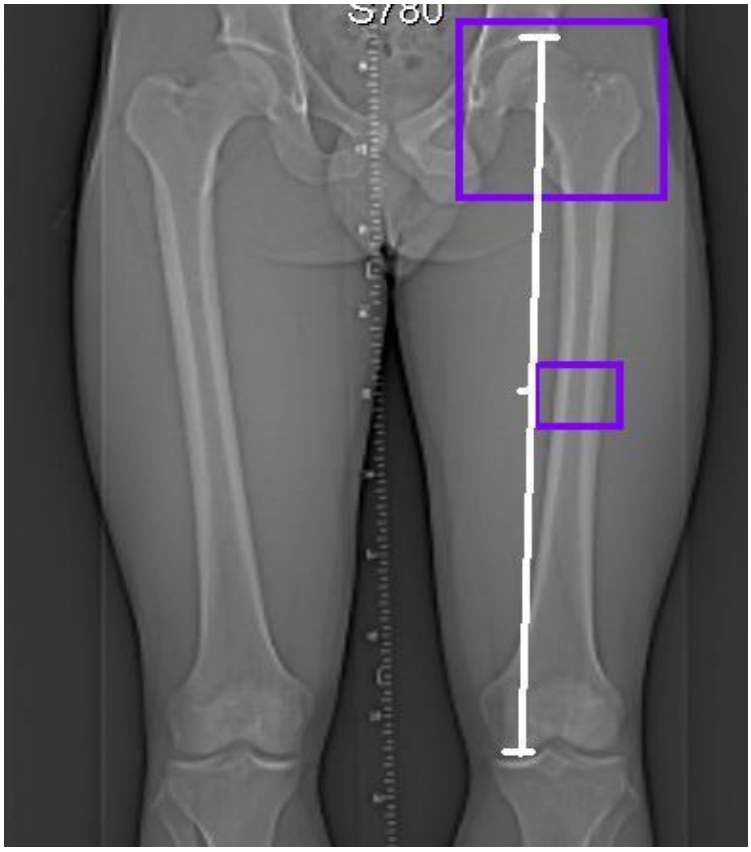
Precision

To test the effect of acquisition, repositioning, processing on segmentation and quantification of the variables quantified by StrAx using HR-pQCT images, seven women had four measurement each. The precision of the StrAx measurements had CV ranged between 0.5-3.0% (40)

Low-resolution clinical computed tomography

Paper II and III

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 (159). 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. 16). 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. 16: *Computed tomography protocol of the proximal femur and femur midshaft scanning.*

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 using the StrAx software (StraxCorp Pty Ltd, Melbourne, Australia), and the collaborators were blinded to the fracture status and diabetes status (40). 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. 17).

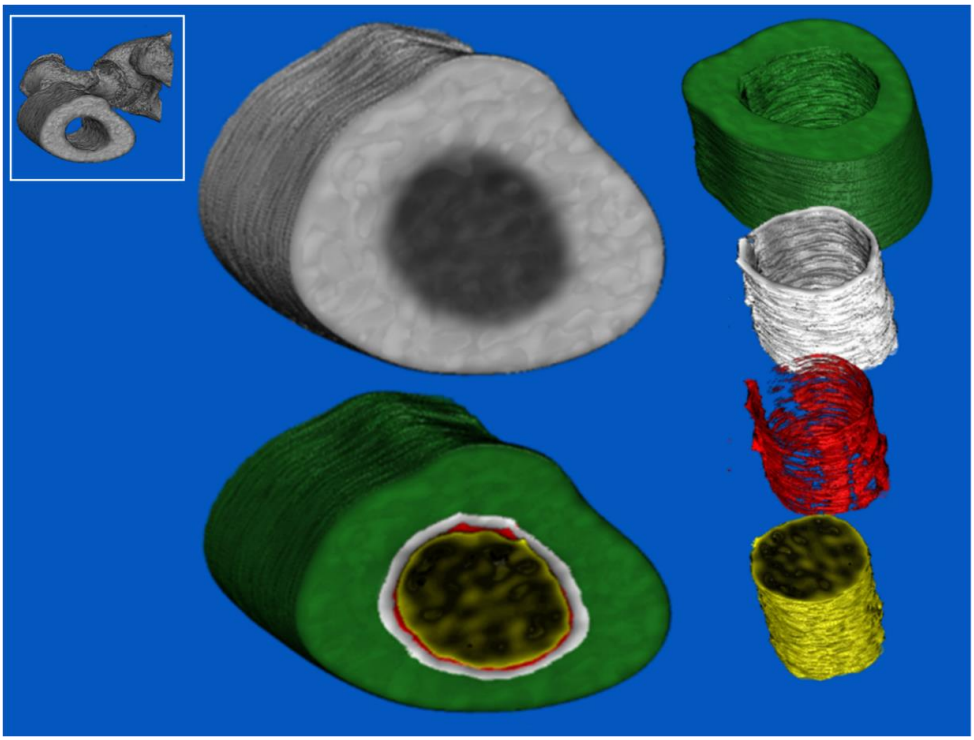


Fig. 17: *Segmented computer tomography image*
Segmented computer tomography image obtained at the femoral subtrochanter using non threshold-based image analysis, showing the total cortical area (the area used for the cortical porosity measurements); consisting of the three cortical compartments: compact appearing cortex (green), the outer (white) and inner (red) transitional zones, and trabecular bone area (yellow). Reprinted from Osteoporosis Int, Ahmed et al. Copyright © 2015, with permission from Springer (159).

The subtrochanteric region within the ROI on CT images was segmented into the compact-appearing cortex, TZs, and trabecular compartment using StrAx software, as in HR-pQCT images (159). This non-thresholding method that automatically selects attenuation profile curves similarly in low-resolution images at the subtrochanteric site (159, 173) as in HR-pQCT images at the distal radius and distal tibia are outlined in detail above and demonstrated in Fig. 13 (40). Of the total cortex at the subtrochanteric site, 70.0% was compact-appearing cortex, while 22.3% and 11.7% were OTZ and ITZ, respectively. 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 (40).

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 (159, 173). 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%) (159, 173). 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%. For ethical reasons, it was not possible to perform *in vivo* validation by rescanning women on the same day, as this would invoke to much radiation. Additional validation of the StrAx 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% (159). This human hip phantom was delivered with the CT scanner (Siemens Somatom Sensation 16, Erlangen, Germany). We presented the following variables: femoral subtrochanteric porosity of the total cortex, compact appearing cortex, and the OTZ and ITZ; total and cortical volumetric BMD (vBMD); trabecular BV/TV; and cortical thickness, which were all quantified using the StrAx software.

3.6 Statistical analyses

Paper I

In paper I, summary statistics are presented as mean and SD. Cases and controls were compared using linear regression analysis for continuous data, and logistic regression analysis for binary data, adjusted for age. The associations of cortical and trabecular morphology (outcomes) as a function of medullary adiposity (exposure) were tested in linear regression models adjusted for age, height, weight and fracture status whenever significant. Odds ratio (OR) for fracture per 1 SD change in medullary adiposity, cortical porosity and other cortical and trabecular bone morphology were calculated in logistic regression analyses adjusted for age (quadratic). In both linear and logistic regression analysis, we used the generalised estimating equations (GEE) method, which took into account the correlations within twin pairs. Distal tibia and distal radius variables were standardised to have mean = 0 and SD = 1 in the linear and logistic regression analysis. Analyses were performed after adjustment for age and FN aBMD, and by combining medullary adiposity and cortical porosity in the same models to test whether they were independently associated with fracture. We tested the interactions between medullary adiposity, cortical porosity and FN aBMD. Sensitivity and specificity for fracture prevalence at the thresholds of medullary adiposity >80th percentile and cortical porosity >80th percentile were explored. Using these thresholds (with all participants as reference), we identified the proportion of women with fracture and controls meeting these criteria, and additional women identified when medullary adiposity was included. Analyses were performed using STATA Software package, v14 (StataCorp, LP, Texas, USA) and SAS software package, v9.4 (SAS Institute Inc., Cary, NC, USA). All tests were two-sided and $p < 0.05$ considered significant.

Paper II

In paper II, data from a prior nested cases-control study were pooled and analysed as a single cohort. The associations were adjusted for fracture status to avoid confounding due to differences between cases and controls. The results are presented stratified by T2DM-status and by fracture status. All normally distributed continuous variables are presented as mean ± 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 variance (ANOVA), 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 (outcomes) as a function of serum glucose and BMI (exposures) are presented. Linear regression analysis was used for associations of BTM and bone architecture (outcomes), as a function of glucose and BMI (exposures) 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.

Paper III

In paper III, differences between fracture cases and controls were assessed using ANOVA and were adjusted for age. The data were pooled, analysed as a single cohort, and adjusted for fracture status to avoid confounding due to differences between cases and controls. The participants were divided into quartiles according to either serum 25(OH)D or serum PTH levels. Differences between women in each of the 25(OH)D and PTH quartiles were compared using ANOVA, and adjusted for age, height, weight, and fracture status. Due to multiple comparisons, we adjusted all p-values in the analysis by controlling the false discovery rate using the Benjamini-Hochberg method (174). Linear regression analysis was used to identify associations between serum 25(OH)D and PTH levels (exposures) with bone turnover markers and bone parameters (outcomes), and the analyses were adjusted for age, height, weight, fracture status, calcium supplementation, corrected serum calcium levels, and the season (winter vs. summer) during which blood sampling occurred. We used standardized regression coefficients to facilitate comparing the strengths of the associations between the exposure and outcomes. The OR for fractures per SD change in serum 25(OH)D and PTH levels were calculated using logistic regression analysis, and were adjusted for age, height, weight, calcium supplementation, corrected serum calcium levels, oral glucocorticoid use, ulcerative colitis or Crohn's disease, and season of blood sampling. They were also mutually adjusted for 25(OH)D and PTH levels, and further adjusted for cortical porosity, cortical thickness, and FN aBMD. A total of 78 fracture cases and 143 controls had the blood samples collected during summer (defined as April-September), while 133 fracture cases and 89 controls had the blood samples collected during the winter season (October-March). Therefore we included season (summer vs. winter) as a covariate in the analysis, and tested whether the association between 25(OH)D and PTH with fracture was modified by season.

4 Main results

Paper I. Combining medullary adiposity and cortical porosity identifies more women with nonvertebral fractures than either measurement alone

Medullary adiposity, cortical and trabecular microarchitecture were quantified at distal tibia and distal radius using HR-pQCT and StrAx software, and FN aBMD using DXA in 79 women aged 40-70 years with nonvertebral fractures, and 345 fracture-free controls in Melbourne, Australia.

Fracture cases had higher distal tibial medullary adiposity and higher cortical porosity. Higher medullary adiposity was associated with higher cortical porosity and higher numbers of thinner and more separated trabeculae. Higher medullary adiposity and cortical porosity were associated with increased odds for nonvertebral fracture (OR [95% CI] were 3.43 [2.24-5.27] and 1.88 [1.23-2.85], respectively), adjusted for age and FN aBMD. Of 77 women with fracture, using a medullary adiposity threshold >80th percentile, 22 women (28.6%) with fracture missed by cortical porosity were identified, while using cortical porosity threshold >80th percentile, 11 women (14.3%) missed by medullary adiposity were identified. The sensitivity was 52.0% using medullary adiposity, 37.7% using cortical porosity, and 66.2% using both, and specificity using both was 77.3%. Results were similar for distal radial measurements.

Thus, a combination of measurements of medullary adiposity and cortical porosity may improve identification of women at risk for nonvertebral fracture.

Paper II. 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

In pooled data based on a prior nested case-control study of 443 postmenopasual women aged 54-94 years from Tromsø 4 (211 fracture cases and 232 fracture-free controls), 22 women had T2DM and 421 did not have diabetes. All had fasting blood samples assayed for PINP, CTX and glucose, and femoral subtrochanteric architecture quantified in low-resolution CT images.

Women with T2DM had higher serum glucose, BMI, and higher femoral subtrochanteric total vBMD, but lower cortical porosity than did nondiabetic women. Increasing serum levels of glucose and BMI were associated with lower serum PINP and CTX. Glucose was associated with lower cortical porosity, while BMI was associated with thicker cortices.

The increasing glucose and BMI that were associated with lower bone turnover markers, is suggesting that reduced intracortical and endocortical remodeling leads to reduced porosity and thicker cortices. Using low-resolution clinical CT, we found lower cortical porosity 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.

Paper III. Serum parathyroid hormone is associated with increased cortical porosity of the inner transitional zone at the proximal femur in postmenopausal women: the Tromsø Study

In a nested case-control study, serum 25(OH)D, PTH, PINP and CTX were measured, and femoral subtrochanteric architecture was quantified in clinical CT images in 211 postmenopausal women aged 54-94 years with nonvertebral fracture, and 232 controls from Tromsø 4. In additional analysis, data were pooled and analyzed as a single cohort.

Women with fractures had lower 25(OH)D, higher PTH, PINP, and CTX than controls, increased femoral subtrochanteric cortical porosity, and reduced cortical thickness and FN aBMD. Serum 25(OH)D was not associated with cortical parameters, or with PINP and CTX. Serum PTH was associated with increased PINP, CTX, and cortical porosity of the inner transitional zone, and reduced trabecular BV/TV and FN aBMD. Decreasing 25(OH)D and increasing PTH were associated with increased odds for fracture independent of cortical porosity and several other covariates.

The results suggest that PTH by inducing increased intracortical bone turnover leads to trabecularization of the inner cortical bone, even in a cohort of relatively healthy postmenopausal women.

5 Discussion

5.1 Methodological considerations

Study design

In paper I we utilized a case control design. Cases were women above 40 years of age, recruited from the ED at the Austin Hospital, Heidelberg, in Melbourne, Australia, within 14 days of suffering a nonvertebral fracture. Controls were women above 40 years of age, recruited from Twins Research Australia. In paper II and III we utilized data from a nested case-control study to compare fracture cases and fracture-free controls, who were recruited from Tromsø 4. In both paper II and III, the data from cases and controls were also pooled and analyzed as a single cohort.

5.1.1 Internal validity

There are three major types of errors that can threaten internal validity: Selection bias, information bias and confounding (175). Most bias can be classified into two major categories: Selection bias and information bias.

Selection bias

Selection bias is a potential threat to the validity of studies. It is presented when individuals have different probabilities of being included in the study sample. This gives a systematic error in the study, that comes from the procedures used to select the subjects in the study, and from diverse factors that can influence their participation (176-178).

Case-control studies are prone to selection bias (178). The challenge for case-control studies is to make sure cases and controls are drawn from the same background population. This is more of a challenge in paper I than in paper II and III. The twin pairs used as controls in paper I are the subjects of a broad study of the genetic and environmental determinants of bone fragility in Melbourne, Australia (65). There is no evidence that twins differ from non-twins in their health, or risk of disease. However, these women have signed up at a registry of the Twins Research Australia because they are willing to give their time to participation in many studies, and they are therefore expected to be more healthy. Moreover, they were recruited from the whole Melbourne region, some of them live outside the city of Melbourne. They may thus differ to

some extent from the fracture cases in paper I who were hospital patients from the region located near the Austin Hospital, in the northeast region of the city of Melbourne. As those who are willing to participate often have better health than those who have problems participating, there may be a healthy selection bias among the women with fractures, as well as in the control group. In paper I, we cannot with certainty state that cases and controls were drawn from the same background population. In contrast, for the Tromsø cohort, cases and controls were recruited from the same Tromsø 4, with a nested case-control design. The relative high attendance rate in the Tromsø Study makes selection bias less of a concern than in studies with low response and attendance rates (165). Nevertheless, "healthy" selection bias, and non-responder bias have to be taken into account. We strived to make sure all eligible cases and controls received the initially invitation letter, followed by one reminding letter to non-responders, and then a pre-screening phone call to check the eligibility in accordance to the inclusion and exclusion criteria, to lower the non-response rate.

In other studies, non-responders and those who do not participate, are reported to have lower socioeconomic status, poorer health, and higher mortality (179-181). After the invitation, we received phone calls from the women who explained that they could not participate in the study due to health issues, and those who had hip prostheses or metal screws, or received treatment for osteoporosis who had to be excluded from participation (due to technical/lab assessment errors). In 760 women with fracture who we invited, a comparison was done between the Tromsø 4 characteristics of 264 fracture cases who participated in the present nested case-control study, with the characteristics of those 496 fracture cases who were invited but did not participate (Table 1) (156). The ones who refrained participation were less physically active, they were 8.1 years older, more of them had experienced a prior hip or wrist fracture, and they had poorer self-perceived health. A tendency towards a "healthy" selection bias was therefore observed in our nested case-control study, so associations between exposure and outcome variables might be underestimated.

Table 1. Characteristics at the Tromsø 4 (1994–95) survey of fracture cases who participated and the fracture cases who were invited but did not participate in this study (of all 760 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 (156).

Information bias

Information bias can occur if there is error in the collection of information from study participants, on exposure and outcome. There might be imperfect definitions of study variables or flawed data collection procedures, which can lead to measurement errors (177, 178). This might in turn lead to misclassification of exposure and/or outcome status for the study participants, and further result in over- or underestimation of associations between exposure and outcome (178). Non-differential misclassification means the study groups, e.g cases and controls, are affected to the same extent, and this error can dilute the association (178). In differential misclassification the rate of misclassification differs between these groups, which can lead to under- and overestimation of associations (177).

Data from questionnaires

In case-control studies one type of exposure identification bias is common, so called recall bias. Since the exposure is assessed after the outcome, participants will often have to remember events that has happened years earlier. Information from the self-administered questionnaires might be prone to bias, resulting in over- or underreporting. In paper I, the fracture cases were recruited within 14 days of having a nonvertebral fracture, to minimize the likelihood that changes in cortical porosity or medullary adiposity was following the fracture. In paper II-III, the index fractures had happened at a median of 6.6 years before the women participated in the

study, however, we used the validated fracture registry as the main source of information about the index fracture. For paper I-III, information about lifestyle factors, as the use of supplementation and the rate of smoking, could have changed in participants experiencing a fracture (e.g taking more supplements, quit smoking). This could also be biased due to difficulties in remembering the information. There could also be a situation where fracture-free controls recall past exposure in less detail than those who have actually fractured. Inaccurate reports of exposure are expected to be more or less similar for controls and cases, and such non-differential misclassification might have diluted true associations.

In paper I, women taking HRT, those with movement artifacts, controls below 40 years of age, and those with prior fracture were excluded, to ensure we had fracture-free controls. In paper II-III, we also excluded some participants for the same reasons, to secure matching. We excluded premenopausal women, those taking HRT and anti-osteoporotic drugs (AOD), as this may influence on the bone architecture, as reduced cortical porosity (173). In addition, we excluded those with movement artifacts, pathological fractures, hip prostheses or metal screws in the hip region after a hip fracture. Metal on one side can cause noise in the CT images at both sites of the hip. Therefore, most of the fracture cases included in the study had fractures of the wrist or the humerus and only few had hip fracture, only those who had the metal removed.

Measurements, and blood samples

Erroneous measurements are sources of information bias. In paper I-III, measurements of height and weight are potential biases where incorrect values may affect the research result. At both study sites, height and weight were measured wearing light clothing and no shoes, to assure measurements were obtained at a standardized setting as accurate as possible by the same research staff, trained to do this. For paper I we did not utilize blood samples, but for paper II-III, analysis of blood samples were included. To avoid erroneous measurements that could bias the results, some precautions were made. BTMs have biological variation, and level of serum glucose varies with food intake. So to limit the errors due to diurnal variation, and effects of meals, the participants came fasting and all had the blood samples collected in the morning, between 8 and 10 AM. Blood samples packed frozen on ice, were transported by plane to Oslo and Bergen. Validated measurement methods were applied for analyses of blood samples.

Samples were assayed for serum glucose with a CV of 0.5-1.6%, insulin with a CV of 0.8-4.6%. 25(OH)D was analyzed using mass spectrometry with a CV of 5%, PTH with CV of 7-12%, and BTMs with CV of 3-8%, respectively. Still, one single measurements of 25(OH)D, PTH, glucose and insulin may not reflect the actual levels throughout the whole study period, so there is a chance of information bias in the blood sample measurements.

Bone measurements

aBMD measurements using DXA

The operator technique might influence the FN aBMD (paper I-III). However, the same DXA machine was used for all measurements in the Tromsø cohort, and same DXA machine was used for the Melbourne cohort. The DXA measurements of aBMD have good precision with CV of 1.2-2.6%.

High-resolution peripheral quantitative computed tomography and StrAx software

In the Melbourne cohort (paper I), bone architecture and medullary adiposity of distal tibia and distal radius was quantified in HR-pQCT images using the StrAx software. Accuracy of porosity measurements at distal radius and distal tibia using HR-pQCT images was validated against µCT images of cadaver specimens (40). Accuracy of porosity quantified at the proximal femur in HR-pQCT images was also assessed against scanning electron microscopy images of specimens as the gold standard (40). The R^2 between HR-pQCT and gold standards ranged from 0.87 to 0.99. The *in vivo* precision of StrAx analysis of HR-pQCT images was tested by rescanning seven women four times, and the *in vivo* and *ex vivo* precision error was <4.0% (40). The chance of measurement error and incorrect measures was therefore limited regarding the utilization of these methods.

Low-resolution CT of the proximal femur and StrAx software

In the Tromsø cohort (paper II-III), bone architecture of the subtrochanteric region was quantified in low-resolution CT images using the StrAx software, as described in the methods section. Validation of the StrAx analyses for the femoral subtrochanter cortical porosity, and the low-resolution CT parameters was performed by repositioning and rescanning a human hip

phantom 10 times (159). CVs were between 0.3 and 2.3%, and regarded as good precision. Accuracy of porosity measurements using CT (voxel size 740 microns) was validated ex vivo against HR-pQCT (voxel size 82 microns), at the same ROI at the femoral subtrochanteric site, in cadaveric specimens (159, 173). Agreement between measurements exceeded 90% (159).

Confounding

A confounder is associated both to exposure and outcome (178), and is responsible for an association (noncausal) between an exposure and the outcome. The confounder is not on the causal pathway between exposure and outcome (178). The confounding variable is not the variable under study. Still, it may influence on the outcome, and result in flawed conclusions about the associations of the exposure and the outcome. As described by Szclo and Nieto a confounding variable (or a group of variables) might weaken, eliminate, strengthen or induce an association between an exposure and a given outcome. In paper I, we are aware that women with fractures were older than controls, and height and weight did not differ between cases and controls. Fracture cases had lower aBMD than controls, and there was a higher percentage of women with osteopenia in the group of women with fracture than without. We adjusted for potential confounders that are known factors associated with bone architecture and fracture, as previously reported in other studies. Associations of cortical and trabecular morphology as a function of medullary adiposity, were in the regression models adjusted for age, height, weight and fracture status whenever significant (164). It is known that with increasing age, especially around menopause for women, remodeling becomes rapid and unbalanced (182). This results in microstructural deterioration, with increased cortical porosity, thinner and more fragmented cortices, and thinner trabeculae, that might eventually disappear. Height, weight and aBMD are well known to be associated with cortical porosity and/or risk for fracture, and we therefore used height, weight, cortical porosity as well as aBMD as covariates when examining the association between medullary adiposity and fracture. In paper II-III, cases and controls were age-matched, the controls were women within the same 5-year age groups. Age is known to be associated with fragility fractures, so we minimized this effect of potential confounding by matching cases and controls by age, and also adjusting for age. In paper II, we examined the associations of BMI and glucose (exposure), with BTMs and bone architecture (outcomes). We adjusted for age and fracture status, that both can affect the outcomes. When we compared groups with and without T2DM, with and without fracture, we adjusted for age and BMI as covariates known to be associated with T2DM and fracture.

In paper III we examined associations of serum 25(OH)D and PTH (exposures) with BTMs and bone architecture (outcomes). Analyses were adjusted for many potential confounders as age, height, weight, fracture status, calcium supplementation, corrected serum calcium levels, inflammatory bowel disease and season of blood sampling. We calculated odds for fractures, per SD change in serum 25(OH)D and PTH and adjusted for age, height, weight, calcium supplementation, corrected serum calcium levels, oral glucocorticoid use, ulcerative colitis, inflammatory bowel disease, and season of the blood sampling. In addition, they were mutually adjusted for 25(OH)D and PTH levels, and also for cortical porosity, cortical thickness, and FN aBMD. As factors that are non-significantly associated with the outcome in bivariate analysis, are known to still become potential confounding factors in a full multivariable model, we kept included both the significant and non-significant covariates in the final models in paper II and paper III. As a total of 78 fracture cases and 143 controls had the blood samples collected in the summer (April-September), while 133 fracture cases and 89 controls had the blood samples collected in the winter season (October-March), we adjusted for seaon (summer vs. winter) as a covariate in the analyses of the associations that involved 25(OH)D and PTH.

Interaction

The effect of an exposure on an endpoint can be modified by a third factor. Interaction occurs when there is difference in the effect of one factor by the level of another factor on the endpoint studied (178).

In paper I, to evaluate whether the association of medullary adiposity and cortical porosity with fracture was modified by aBMD, we included interaction terms in the logistic regression model but found no interaction between the variables. We are still aware that interaction might be difficult to detect, and that interaction is not totally excluded solely because of lack of a significant interaction term. In paper III we wanted to evaluate whether the association of 25(OH)D and PTH levels with odds for fracture was modified by season. Therefore we included interaction terms between 25(OH)D and PTH levels, and the season of blood sampling (summer vs. winter) but found no interaction between the variables.

5.1.2 External validity

External validity, or generalizability, is the extent to which results of a study are generalizable to the source population as well as other populations. We studied two different cohorts. The cohort in paper I was composed of women who presented to a hospital in Melbourne, Australia, after suffering a nonvertebral fracture, and controls were relatively healthy women recruited from the Twins Research Australia. All women were Caucasian and above 40 years of age. The fracture case group was small, which may have affected the representativeness. The control group was larger, and we can believe that this group of controls are representative for Caucasian women in big cities in Australia and Europe, and other countries with inhabitants of Caucasian origin. The cohort in paper II-III was drawn from the Tromsø Study. The whole population of Tromsø was the source of invitations to the participants. The study participant were Caucasian postmenopausal women aged 54-94 years old. Tromsø is located above the Arctic circle, almost at 70°N. The sun is below the horizon for 2 months during the winter season, and dermal vitamin D synthesis can be absent for up to 5 months (183, 184). Despite this, in population based studies, people in Scandinavia are not reported to have lower levels of 25(OH)D than those in other European countries (163, 185). In Norway, differences in fracture risk between the population living in urban versus rural areas have been reported (186). Mainly, the population of Tromsø may be considered representative of both the Norwegian, and the European population, with the same age, gender and ethnicity.

5.2 Significance of the results

5.2.1 Medullary adiposity, cortical porosity and nonvertebral fractures

We found higher medullary adiposity to be associated with higher cortical porosity of each cortical compartment, particularly the inner transitional zone. The association of medullary adiposity with higher porosity of the compact cortex is of particular interest, since less bone mass in this bone region, located further from the neutral axis, may reduce the bone strength to a larger extent than less bone mass (higher porosity) in the inner cortex (30). We infer that this may contribute to explain the increased fracture risk associated to higher medullary adiposity.

In the current study, women with nonvertebral fractures had higher medullary adiposity than controls. Medullary adiposity was associated with prevalent nonvertebral fractures, independent of aBMD and cortical porosity. To the best of our knowledge, an association between medullary adiposity and nonvertebral fracture has not been reported previously. It is consistent with previous reports, where higher vertebral marrow fat was associated with compression fractures independent of vBMD (141).

We calculated medullary adiposity score as a function of the fat proportion and the relative medullary density in the medullary cavity at the distal tibia and distal radius using HR-pQCT. The medullary density becomes more negative with the increase of fat cells, so lower density means more yellow fat tissue. There are few papers published based on pQCT for assessment of marrow adiposity (187). One study using pQCT found tibial marrow density, as an estimate of marrow adiposity, lower in premenopausal female athletes with a long history in sports than in physically active, healthy, but nonathletic controls (187). They suggested that loading history is a predictor of tibial bone strength. Previous studies have mostly used MRI and measured vertebral bone marrow adiposity (142), and reported that osteoporosis were associated with lower unsaturation levels (157, 188), and higher saturation levels of marrow fat, using MRS (157). Although there are differences in measurement methods, measurement sites, and fracture sites, as well as differences in ways of estimating marrow adiposity, there is a growing consensus that changes is marrow adiposity are linked to adverse changes in skeletal metabolism (43, 143).

For the combination of medullary adiposity and cortical porosity the sensitivity for fracture was 66%, and specificity was 77%. This high sensitivity sounds promising, although the specificity is limited. Assessing the potential benefit from a measurement of marrow adiposity remains a challenge due to the case-control design and limited sample size as described in the methodological section above.

5.2.2 Type 2 diabetes mellitus and cortical porosity

In paper II, we reported that women with T2DM had lower cortical porosity of the subtrochanteric region than did women without diabetes. PINP tended to be lower in those with T2DM, but this was not statistically significant. We found increasing glucose and BMI to be associated with lower PINP and CTX. Furthermore, increasing glucose was associated with reduced cortical porosity.

Although T2DM is a modest risk factor for fracture, the diabetic patients will benefit from clinicians being aware of this risk. Fragility fracture is a clinical concern in those with T2DM due to the high number of subjects with this disease (126). After exploring medullary adiposity and fracture, we wanted to explore T2DM, a condition where subjects might be prone to altered metabolic function, and where marrow adiposity has been reported to be increased (46, 118). T2DM subjects tend to have higher BMI, and higher aBMD than nondiabetic subjects do. In healthy subjects, a higher aBMD would be protective against fracture. Nevertheless, in T2DM patients, fracture risk is higher for a given level of aBMD (112), and the reasons for this are under current investigation, and it is likely to be multifactorial (110, 189).

We were aware that cortical porosity had been invoked as a possible factor responsible for fractures in the T2DM patient group, measured in the distal tibia and distal radius, using HR-pQCT (119-121). However, since increased cortical porosity reflects increased bone turnover, whereas T2DM is a low bone turnover condition, we expected T2DM patient to have reduced cortical porosity. We explored this in our data, in a small sample of 22 women with T2DM, from the Tromsø cohort, utilizing clinical CT at the proximal femur, and the StrAx software.

The proximal femur is a common site of the most serious fragility fracture, so we wanted to examine porosity here, since it is not clear whether measured porosity of peripheral sites are representative for central sites. We found cortical porosity to be lower in T2DM subject than in the 421 women without diabetes. This was not in line with previous reports, but we confirmed the findings from a study published around the same time as paper II, which reported lower cortical porosity at the distal radius inn 99 women with T2DM, compared to 954 controls without diabetes using HR-pQCT (124).

A small pilot study stated higher cortical porosity in T2DM subjects than in controls. They discussed that this result could be driven by the fact that two subjects in the T2DM group had previous fractures (121). The fracture subjects had high levels of cortical porosity of distal tibia and distal radius. When these subjects were excluded from the analysis, the authors reported that the difference in porosity was reduced (121). Another study reported higher cortical porosity in T2DM subjects with fracture, than in T2DM subjects without fracture (significant at ultradistal tibia, not significant at ultradistal radius) (190). Most probably, the higher porosity reflects the fact that the subjects had suffered a fracture. When comparing fracture-free T2DM subjects with healthy fracture-free controls, subjects with T2DM had lower cortical porosity of both distal radius and distal tibia than those without T2DM, without reaching statistical significance (190). Our findings are in agreement with the non-significant trends in this paper.

Another novel finding in this paper is that higher serum glucose was associated with lower BTMs and with cortical porosity in the pooled data consisting of 443 subjects. This finding support the hypothesis were we tested whether women with the low-bone-turnover-condition T2DM have lower cortical porosity. Moreover, so did the finding that higher BMI was associated with lower BTMs and thicker cortices. Despite the low number of subject with T2DM, we do believe that our findings are contributing to increased understanding of these associations, even though a lot still remains to be explained.

We further illustrate that fracture cases have higher cortical porosity (which fits with high bone turnover), while T2DM cases tend to have lower cortical porosity (which fits with low bone turnover). We hereby illustrated these trends in subgroups where we compared women with and without T2DM and women with and without fracture, as done in a previous study (190).

Taking these results together, we inferred that cortical porosity is unlikely to explain the increased fracture risk in women with T2DM, but probably other alterations in bone qualities are more likely to be responsible for this risk. High glucose levels result in a process, nonenzymatic glycation, that again may lead to accumulation of advanced glycosylation end-products (AGEs) in bone matrix (127). AGE crosslinks gives more brittle bone with less toughness that easier may fracture (128). AGEs have also been reported to decrease bone formation, by interfering with osteoblasts (127), and bone turnover has been reported reduced in T2DM subjects (117, 118). In the current study, bone turnover markers were not significantly lower in those with T2DM, but increasing glucose and BMI were associated with lower PINP and CTX. Both structural and material abnormalities have been suggested to be responsible for increased fracture risk in T2DM subjects (127). In contrast to some previous reports, we found that T2DM subjects had lower cortical porosity, as did one other study (124, 189). Structural and material abnormalities can be intertwined, with abnormally mineralized collagen. Glycated collagen matrix can lead to compromised biomechanical function (127). It is important for clinicians to be aware that the most commonly used tool, DXA measurement of aBMD, cannot assess the material and structural determinants of bone strength.

5.2.3 Serum parathyroid hormone and cortical porosity

In paper III we explored associations between PTH, 25(OH)D, cortical porosity and fracture risk, in the Tromsø cohort. We confirm that decreasing 25(OH)D and increasing PTH are associated with increased odds for fracture. However, in contrast to our hypothesis, these associations were independent of cortical porosity. PTH, not 25(OH)D was associated with increased bone turnover markers and higher cortical porosity of the inner transitional zone at the proximal femur.

Low serum levels of 25(OH)D are associated with secondary hyperparathyroidism and risk for fracture (131-133, 162, 163), but there has been few reports exploring the role of cortical porosity in this association. Therefore, whether fracture risk associated with low 25(OH)D and high PTH is mediated through increased cortical porosity remains less clear (191, 192).

Tromsø is located far north, the sun is below the horizon for 2 months during winter, and dermal vitamin D synthesis can be absent for up to 5 months. This is one of the reasons for why there has been interest in studying vitamin D in relation to bone health using DXA, and we wanted to explore 25(OH)D in relation to 3-dimensional bone architecture, quantified by StrAx from clinical CT images.

Lower serum 25(OH)D and higher PTH were independently associated with fractures after mutual adjustment for cortical porosity and thickness. We expected low 25(OH)D and high PTH to be associated with fracture, mediated by cortial porosity, but they were both independently associated, after mutual adjustment. The effect of low 25(OH)D and high PTH on fracture risk, can still be mediated through porosity because the current study has limitations as discussed in the methodological discussion section above. Our cohort of women was of a moderate sized retrospective case-control study with only one measurement of 25(OH)D, PTH and BTMs, with normal levels of 25(OH)D. In women with fracture, serum 25(OH)D levels were lower, and PTH and the bone turnover markers PINP and CTX were higher. Increasing PTH, not decreasing 25(OH)D, was associated with increased bone turnover markers, and increased cortical porosity of the inner transitional zone, resulting in trabecularization of the inner cortical bone. 25(OH)D pointed in the direction of an increase in porosity, as well as thinner cortices, with decreasing 25(OH)D, but this was not statistically significant. A lack of association between 25(OH)D and bone turnover markers and cortical porosity may be due to the small proportion of subjects with low 25(OH)D < 50 nmol/L (10.9% of cases and 11.6% of controls). This may reflect a lack of statistical power in the analyzes to detect small effects. To show a potential association, we might need a larger number of subjects with 25(OH)D deficiency.

6 Conclusions, implications, and further research

6.1 Conclusion

Medullary adiposity was associated with more porous cortices and deteriorated trabecular microstructure of distal tibia and distal radius, and higher odds for detecting women with nonvertebral fracture independent of cortical porosity and bone mineral density of the femoral neck. Additional women with prevalent fracture were identified when medullary adiposity was combined with cortical porosity than by either measurement alone.

Subjects with T2DM are known to have increased risk for fracture. According to our data, it is not due to an increase in cortical porosity as has been stated in other publications. Increasing cortical porosity is thus unlikely to explain the increased fracture propensity in postmenopausal women with T2DM. We infer that other factors, as alterations in bone material composition or increased microdamage due to reduced bone turnover might rather contribute to their increased fracture risk.

Serum PTH, not 25(OH)D, is associated with increased intracortical bone turnover, resulting in trabecularisation of the inner cortical bone. Decreasing 25(OH)D and increasing PTH are associated with fracture risk, independent of cortical porosity and thickness.

6.2 Implications and further research

Prospective studies of sufficient sample size are needed to determine whether a measurement of medullary adiposity assessed using pQCT is of benefit to identify additional women sustaining incident fracture, than those identified by assessment of bone morphology and bone mineral density. In addition, further development of methods to improve the sensitivity for prevalent and incident fracture remains a challenge. Work on medullary adiposity, to find the contribution of fat proportion and fat density, to best identify if these are risk factors for fracture, or can predict fracture, is still important.

There is an ongoing debate whether cortical porosity is increased or decreased in subjects with type 2 diabetes mellitus. Larger prospective studies are needed to confirm that cortical porosity is lower in T2DM patients. Confirming lower porosity in these patients, and manifesting the knowledge that T2DM is a low bone turnover condition might help to clarify the pathophysiology behind fracture risk in these subjects. This might have clinical implication regarding treatment, since these patients often are offered antiresorptive medication today.

Further work is needed to determine the role of cortical porosity in the association of serum 25(OH)D and PTH with fracture risk in individuals with low levels of 25(OH)D and in a larger sample of individuals, to understand the mechanism for how these hormones may influence cortical bone architecture and the risk for fractures.

The field of bone fragility needs efficient tools for identification of women who are at high risk for fracture, so these can be offered treatment, without treating those who do not need it. To develop better tools for identification of individuals at high risk for fracture, we need to improve our understanding of the mechanisms behind the increased fracture risk, and the pathophysiology behind the associations between measurements, that are available with new technology. Further research must move in the direction of identifying these mechanisms in basic scientific studies as well as in epidemiologic studies with proper sample size and design. This could provide evidence for what works well in a real clinical setting with patients.

7 References

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Paper I

Paper II

Paper III

Appendix A

Questionnaire, Australia

Main Questionnaire B – to be filled in when the tests are done

Bone Structure and Strength during Menopause: a co-twin control study

First name:_____Middle Name_____Surname_____ Current Age ___

Home Address:_____State_____Postcode_____

Tel:_____ (Home) _____ (Work) _____(Mobile)

Personal Doctor:_____ Tel_____

Address_____Postcode_____

Date of Birth:____/____/_____ Date / Month / Year

Date of filling in this form:_____/_____/_____ Date / Month / Year

Country of birth: _____ ____ Ethnicity: Caucasian / Asian / ATSI / Indian / other

Height and weight

How tall are you? _____cm what is your current weight? _____kg

For assessment of zygosity (these 3 questions are relevant for twins only)

1. Do you and your twin sister have the same eye colour (Yes, No)
2. Do you have similar height, weight, complexion and natural hair colour and texture? (Yes, No)
3. Were you usually mistaken for one another by non-family members as children? (Yes, No)

Reproductive History

Please circle Yes or No, do not guess any answers. Indicate if you do not know, or are not sure.

Pregnancy

1. Have you **ever** been pregnant? (Yes, No) If No go to Question 2.
   If Yes, are you currently pregnant (Yes, No) If Yes, in which trimester are you now? ___

   How many live births and stillbirths have you had? ___ How many twins/multiple births? ___
   How many miscarriages, ectopic pregnancies or induced terminations have you had? ___

   How **old** were you with your **first** pregnancy? ____ years
   How **old** were you with your most **recent** pregnancy? ___ years

   If you breast-fed, for how many months did you breast-feed your **first** baby? ____ months
   For how many months did you breast-feed your babies? (Add up for **all**) ____ months

Menstruation and Menopause

2. How old were you when you had your **first** menstrual period? ____ years
Are your periods **regular**? (Yes, No) Date when your last period started ___/____/_____
If your have regular periods, go to question 5. Otherwise, go to question 3.

3. Have you had a period in the last 12 months? (Yes, No) Date for last period ___/___/_____
Have your periods changed in frequency from regular to irregular? (Yes, No)
If irregular, how long is the interval between the current periods? __ months __ weeks or __ days

4. Have your periods stopped completely? (Yes, No) If Yes, how old were you? ____ years
Why did your menstrual periods stop?
Natural menopause (periods stopped by themselves) (Yes, No)
Hysterectomy (womb or uterus removed) (Yes, No)
Both ovaries removed (Yes, No)
Radiation or chemotherapy (Yes, No)
Strenuous exercise (Yes, No)
Illness (Yes, No)
Pregnancy or breastfeeding (Yes, No)
Other reasons, specify (Yes, No)_____

5. Have your periods stopped for more than 2 months when you were not pregnant? (Yes, No)
If Yes, how many times has this occurred? _____
On average how long did each episode last? ____ months
What has been the major reason?_____

Birth Control and Hormone Treatment

6. Have you **ever** used birth control pills or hormonal contraceptives? (Yes, No) _____
Are you currently using birth control pills, hormonal contraceptive implants or injections? (Yes, No)

How old were you when you first used birth control pills or hormonal contraceptives? ____ years
How old were you when you last took birth control pills or hormonal contraceptives? ____ years
For **how long** in total have you taken these? (Add up all the times) ___ months ___ years

Please write the name(s) of any pills or contraceptives you can remember having ever used.

_____

_____

7. Have you had hormone treatment for flushes or other menopausal symptoms? (Yes, No)
Are you currently using hormone treatments for menopause? (Yes, No)

How old were you when you first used hormone treatment for menopause? ____ years
How old were you when you last took hormone treatment for menopause? ____ years
For how long in total did you use this treatment? ____ months ___ years

Please write the name(s) of oestrogen, progesterone or other hormones used for menopause

_____

_____

Medical History

Have you ever had any of the following medical conditions? If No, leave blank.
If Yes, write your age when you were **first diagnosed** with the condition and describe it below;

Osteoporosis	_____	Diabetes	_____
Osteogenesis Imperfecta	_____	Thyroid disease	_____
Paget's disease	_____	Lung disease	_____
Rheumatoid arthritis	_____	Prolonged bed confinement	_____
Epilepsy	_____	Ovarian cancer	_____
Malabsorption	_____	Breast cancer	_____
Milk intolerance	_____	Other cancer	_____

Please specify these or other medical problem

_____

Breast and Ovary Removal or Hysterectomy

9. Have you had a complete removal of one or both breasts? (Yes, No) How old were you? __ Yrs
Why did you have your breasts removed? To treat breast cancer (__)
To prevent getting cancer in that breast (___)
Other, specify_____

10. Have you ever had one (Yes, No) or both (Yes, No) ovaries removed? At what age? ___ Yrs
Why did you have your ovaries removed? To treat ovarian cancer (___)
To prevent getting cancer in that ovary (___)
As part of treatment for breast cancer (___)
As part of prevention of breast cancer (___)
Other, specify _____

11. Have you had a hysterectomy, removal of the womb or uterus? (Yes, No) At what age? __ Yrs
Why did you have the hysterectomy? Uterine cancer (___)
Uterine fibroid (___)
Abnormal uterine bleeding (___)
Cancer prevention (___)
Other, specify_____

Medication

Have you ever taken tamoxifen? (Yes, No) Are you currently taking tamoxifen? (Yes, No)
At what age did you start using tamoxifen? ___ yrs, at what age did you stop using it? ___ yrs
For how long did you take tamoxifen, in total? ___ months, ___ yrs
Did you take tamoxifen to treat breast cancer? (Yes, No), or to prevent breast cancer? (Yes, No)

Have you ever taken raloxifen or Evista? (Yes, No) Are you currently taking this? (Yes, No)
At what age did you start using this treatment? ___ yrs, at what age did you stop using it? ___ yrs
For how long did you take raloxifen or Evista, in total? ___ months, ___ yrs
Did you take raloxifen to prevent breast cancer? (Yes, No), or use it for osteoporosis? (Yes, No)

Do you use supplementation of calcium (Yes / No) Vitamins (Yes/ No) If yes, which?_____
Please list other medications you have used or currently use:

_____

Fractures

Have you ever had a bone fracture? (Yes / No)
If yes please fill in the following details for each fracture.

Bone	(right/left)	Age	How was it broken
_____		_____	_____
_____		_____	_____
_____		_____	_____

Identification of Relatives who have had fracture

Relation	First name	Age	Ever had fracture
Mother:	_____		
Father:	_____		

Lifestyle Questions

1. On average, how many cups of coffee do you drink per day __ cups or per week __ cups

   How much milk do you usually take with your coffee (please circle one)

   None A little (< 2 teaspoons) Some A lot

2. On average, how many cups of tea do you drink per day __ cups or per week __ cups

   How much milk do you usually take with your tea (please circle one)

   None A little Some A lot

4. Had you ever been a regular cigarette smoker? (Yes, No)_____

   Note: regular smoker is at least one per day for 3 months or longer.
   If Yes: At what age did you start to smoke cigarettes regularly? _____years

   Are you currently smoking regularly? (Yes/No)
   On average, how many cigarettes per day do you smoke?_____cigarettes

   At what age did you STOP smoking? _____ years

   When smoking regularly, how many cigarettes did you usually smoke per day? ____ cigarettes

   In total for how many years have you been a regular smoker? _____years
   (Add up all the periods during which you have smoked regularly)

4. Had you consumed alcoholic drinks? (Yes / No)_____
If Yes: At what age did you start to drink alcohol?_____years

For each form of beverage, on average how many standard glasses would you drink in a week?

Beer_____ Wine_____ Spirits_____ Others (specify)_____

Note: For beer a standard glass is 7 oz., for wine it is 4 oz., and for spirits it is about 1 oz.

Have you drunk alcohol (beer, wine or spirits) regularly, that is at least once per week for six months or longer? (Yes, No) Are you currently drinking alcohol at least once per week? (Yes, No)

How old were you when you started drinking alcohol at least once per week? _____ years
At what age did you STOP drinking alcohol at least once per week? _____ years
For how many years or months did you drink alcohol at least once per week? __ years __ months

5. Indicate by a cross (X) how often you eat or drink each food or beverage.

Food or Beverage	Never or Rarely	1-3 times a month	Times a week 1	2-3	4-6	Times a day 1	2-3	4 plus
Milk	___	___	___	___	___	___	___	___
Breakfast Cereal	___	___	___	___	___	___	___	___
Yoghurt	___	___	___	___	___	___	___	___
Cottage Cheese	___	___	___	___	___	___	___	___
Other Cheese	___	___	___	___	___	___	___	___
Eggs	___	___	___	___	___	___	___	___
Cream	___	___	___	___	___	___	___	___
Grapes	___	___	___	___	___	___	___	___
Dried Fruit	___	___	___	___	___	___	___	___
Oranges	___	___	___	___	___	___	___	___
Lemons	___	___	___	___	___	___	___	___
Dried Beans	___	___	___	___	___	___	___	___
Lentils	___	___	___	___	___	___	___	___
Cakes	___	___	___	___	___	___	___	___
Biscuits	___	___	___	___	___	___	___	___
Salmon	___	___	___	___	___	___	___	___
Sardines	___	___	___	___	___	___	___	___
Fish Paste	___	___	___	___	___	___	___	___
Oysters, Scallops	___	___	___	___	___	___	___	___
Tuna	___	___	___	___	___	___	___	___

6. Over the last week:

How many glasses of MILK did you drink? _____ glasses

How much milk did you consume with breakfast cereals? _____ cups

How much YOGHURT did you consume? _____ gms
(Small containers usually 200-250 gms; large ones are about 500 gms)

About how much CHEESE did you eat? _____ gms
(One slice is approximately 20 gms; one 2.5 cm cube is about 30 gms)

7. Over the last few years how often have you sunbaked? (Please circle one)

Never Rarely Sometimes Often Very Often

Over the last few years, whenever you have been in summer sunshine how much skin protection (hat, sunscreen, clothing) do you usually have?

None A bit A fair amount A great deal

As a child how often did you sunbake? (Please circle one)

Never Rarely Sometimes Often Very Often

When unexposed in the summer sun, how easily do you burn?

Very easily Quite easily Not easily Hardly ever

8. What is your current occupation? _____

What has/have been your main occupation(s) during your working life?

_____

In your main occupation (s), how would best describe each of the following physical aspects of your works? (circle one for each aspect)

Sitting:	Never	Seldom	Sometimes	Often	Always
Standing:	Never	Seldom	Sometimes	Often	Always
Walking:	Never	Seldom	Sometimes	Often	Always
Lifting loads:	Never	Seldom	Sometimes	Often	Always

9. On average, on how many occasions per month do you participate at each of the following levels of activity or sport (Answer for each level)

Light (e.g. walking, lawn bowls, light gardening) _____days per month

Moderate (e.g. social tennis, golf, hiking) _____days per month

Vigorous (e.g. competitive active sports) _____days per month

Thank you for your kind help in completing this questionnaire

Appendix B

Questionnaire, Norway

01-2011 Beinstruktur
01.11.2011

Dato ☐☐.☐☐.2 0 1☐ Løpe nr ☐☐☐☐☐

Initialer ☐☐☐☐

HELSE, BRUDD OG SYKDOM

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

☐ Meget god

☐ God

☐ Verken god eller dårlig

☐ Dårlig

☐ Meget dårlig

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

☐ Ja ☐ Nei

Hvis JA, hvor mange brudd har du hatt?

Antall brudd ☐☐

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

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

☐ Nei

☐ Ja, 1-2 ganger

☐ Ja, 2-5 ganger

☐ Ja, mer enn 5 ganger

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

Antall ganger falt ☐☐

Har noen av dine foreldrene hatt lårhalsbrudd:

☐ Mor ☐ Far ☐ Ingen

11061

Løpe nr ☐☐☐☐☐

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

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

.....

.....

RØYKING OG ALKOHOL

Har du røykt/røyker du daglig?

☐ Ja, nå

☐ Ja, tidligere

☐ Aldri

Hvor mange år til sammen har du røykt daglig?

Antall år ☐☐

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

Hvor ofte drikker du alkohol?

☐ Aldri

☐ Månedlig eller sjeldnere

☐ 2-4 ganger pr. måned

☐ 2-3 ganger pr. uke

☐ 4 eller flere ganger pr. uke

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

☐ 1-2

☐ 3-4

☐ 5-6

☐ 7-9

☐ 10 eller flere

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

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Takk for hjelpen!

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