Determinants of Peak Bone Mass in Men

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Cover illustration: Image of ultradistal tibia assessed by HR-pQCT (XtremeCT, Scanco Medical AG, Brüttisellen, Switzerland).

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ABSTRACT

Objective: The aim of this thesis was to identify, investigate and evaluate hereditary and environmental factors associated with peak bone mass or bone development in men.

Method: All studies in the thesis were performed within a well-characterized population-based cohort of 1068 men between 18 to 20 years of age at baseline (the Gothenburg Obesity and Osteoporosis Determinants (GOOD) study). Measurements of bone mass, bone geometry, microstructure and estimated bone strength were assessed using dual-energy X-ray absorptiometry (DXA), peripheral quantitative computed tomography (pQCT), and high-resolution pQCT with applied finite element analysis. A self-administered questionnaire was used to collect information about physical activity, calcium intake, smoking and fracture prevalence. For evaluation of heredity and maternal factors, various Swedish registers were used, and fracture prevalence was verified in local hospital X-ray records.

Results: Family history of a grandfather with hip fracture was associated with reduced areal bone mineral density (aBMD) and cortical bone size in 19-year-old men. Advancing maternal age was a negative predictor of lumbar spine aBMD in 19-year-old men, independently of the possible confounders known to affect bone mass in late adolescence. Young men who started to smoke in young adulthood developed lower aBMD at several sites as well as lower trabecular density and smaller cortical cross-sectional area, than their nonsmoking peers. Prevalent fractures in young adult men were associated with impaired trabecular microstructure at the radius, independently of aBMD and cortical thickness.

Conclusion: We identified heredity over two generations, high maternal age, smoking and prevalent fractures as predictors of low peak bone mass. We suggest that these factors could possibly affect the risk of osteoporosis and fracture later in life.

Keywords: peak bone mass, bone mineral density, bone geometry, microstructure
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SAMMANFATTNING PÅ SVENSKA

Frågeställning: Den huvudsakliga frågeställningen för avhandlingen var att identifiera, undersöka och utvärdera ärftliga och miljömässiga faktorer relaterade till den maximala benmassan (peak bone mass) och utvecklingen av denna hos män. Den maximala benmassan uppnås i ung vuxen ålder vid olika tidpunkter beroende på lokalisation i skelettet.

Metod: Delstudierna i avhandlingen är samtliga utförda inom en välkaraktäriserad populationsbaserad kohort bestående av 1068 unga män mellan 18 och 20 år vid studiestart (the Gothenburg Obesity and Osteoporosis Determinants (GOOD) study). För utvärdering av benmassa, bengeometri, benets mikrostruktur samt beräknad benhållfasthet, användes röntgenbaserad undersökningsutrustning såsom dubbelfotonröntgen absorbtiometri (DXA), perifer kvantitativ datortomografi (pQCT) samt högupplöst pQCT med tillämpning av finita elementmetoden. Information om fysisk aktivitet, kalciumintag, rökningsvanor samt förekomst av tidigare fraktur insamlades med hjälp av ett standardiserat frågeformulär. För utvärdering av ärftlighet, mödrafaktorer, och rapporterade frakturer användes information från flera av socialstyrelsens register samt lokala röntgenarkiv.

Resultat: Studierna visade att ärftlighet var påvisbar över två generationer, där en höftfraktur hos morfar eller farfar var associerad med låg benmassa och mindre kortikal benstorlek hos unga vuxna män. Hög ålder hos modern var associerat med lägre benmassa i ländryggen hos deras unga vuxna söner, oberoende av andra kända riskfaktorer. Debut av rökning mellan 19 och 24 års ålder var förenat med försämrad utveckling av benmassa, med påverkan på både kortikalt och trabekulärt ben. Förekomst av tidigare fraktur var relaterad till lägre benmassa till följd av mindre fördelaktig trabekulär mikrostruktur samt ett mindre kortikalt ben hos unga vuxna män.

Slutsatser: Vi identifierade ärftlighet över två generationer, hög mödraålder, rökning och prevalent fraktur som riskfaktorer för låg benmassa hos unga män. Möjligen kan dessa faktorer ha betydelse för uppkomsten av osteoporos och frakturer senare i livet.

LIST OF PAPERS

This thesis is based on the following studies, referred to in the text by their Roman numerals (I-IV).

I. Rudäng, R*, Ohlsson, C*, Odén, A, Johansson, H, Mellström, D, Lorentzon, M. Hip fracture prevalence in grandfathers is associated with reduced cortical cross-sectional bone area in their young adult grandsons. the Journal of Clinical Endocrinology and Metabolism, March 2010, 95(3): 1105-1114.
* contributed equally

II. Rudäng, R, Mellström, D, Clark, E, Ohlsson, C, Lorentzon, M. Advancing maternal age is associated with lower bone mineral density in young adult male offspring. Osteoporosis International, 2012, 23:475-482.

III. Rudäng, R, Darelid, A, Nilsson, M, Nilsson, S, Mellström, D, Ohlsson, C, Lorentzon, M. Smoking is associated with impaired bone mass development in young adult men: A 5-year longitudinal study. the Journal of Bone and Mineral Research, Vol. 27, No. 10, October 2012, pp 2189-2197.

IV. Rudäng, R, Darelid, A, Nilsson, M, Mellström, D, Ohlsson, C, Lorentzon, M. X-ray verified fractures are associated with finite element analysis derived bone strength and trabecular microstructure in young adult men. Manuscript

CONTENT

ABBREVIATIONS

aBMD	areal bone mineral density
BMC	bone mineral content
BMD	bone mineral density
BMI	body mass index
CI	confidence interval
CSA	cross-sectional area
CV	coefficient of variation
DXA	dual energy X-ray absorptiometry
FRAX®	WHO web-based fracture risk assessment tool
GOOD	Gothenburg Osteoporosis and Obesity Determinants
GWAS	genome wide association study
HT-pQCT	high-resolution peripheral quantitative computed tomography
ICD	International Classification of Diseases
MRI	magnetic resonance imaging
PBM	peak bone mass
pQCT	peripheral quantitative computed tomography
QCT	quantitative computed tomography
RCT	randomized controlled trial
SD	standard deviation
SEI	socioeconomic index

SSI	strength strain index
μSv	microsievert
vBMD	volumetric bone mineral density
VOI	volume of interest
WHO	World health organization

DEFINITIONS IN SHORT

Peak bone mass	The amount of bony tissue present at the end of skeletal maturation.[1]
Osteoporosis	A disease characterized by low bone mass and microarchitectural deterioration of bone tissue, leading to enhanced bone fragility and a consequent increase in fracture risk.[2]

INTRODUCTION

The skeleton

The skeleton is constituted by bone, which is an organ with several functions in the body of mammals. The most obvious task for bones is to support the body, and work as levers for muscles, to enable the body to manage the forces of gravitation and facilitate locomotion. Bones also provide protection for vital organs, e.g. the brain in the skull and the heart in the thorax, and are the primary site of hemopoiesis, which takes place in the bone marrow cavity. Furthermore, the skeleton is a reserve for ions; of which the most essential are calcium and phosphate, and thereby has an important role in homeostasis.[3,4]

Bone structure

There are several ways to categorize bone. In vertebrates, one way is to subdivide the skeleton into axial and appendicular parts, where the axial skeleton includes the skull, spine, sternum and ribs, and the appendicular skeleton includes the bones of the extremities.[3] Another way to categorize is by the gross morphology of the bone. Using this categorization there are principally two major groups of bones: flat bones (scapula, skull, pelvis, ribs, sternum) and long, or tubular bones (limb bones and vertebral bodies). Virtually all bones are organized with a compact and thin outer surface called cortex or cortical bone, and an inner region, which is braced by narrow plates as a meshwork called the trabecular or cancellous bone.[4,5] On the microstructural level the cortical bone is composed by lamellae which are concentrically arranged around a centrally situated canal. This forms a unit called a Haversian system or an osteon, and the central canal is called a Haversian canal. Between the lamellae bone cells called osteocytes are embedded, lying in cavities called lacunae, which in turn are connected with each other and the Haversian canals by canaliculi. In cross-section, this gives the cortical bone a porous appearance, and the volume fraction of pores in the cortical bone are referred to as cortical, or intracortical porosity, which increases with age in both men and women, and correlates well with the natural decrease in bone density in adults.[4,6] The cortical bone is the major contributor to adult bone mass, corresponding to a total of 80% of the adult human skeleton. The proportion of cortical and trabecular bone varies throughout the skeleton depending on site, where, for instance, the vertebral

bodies are composed of about 2/3 of trabecular bone, whereas the mid-forearm is composed of more than 95% cortical bone.[4,7]

Bone biology

Bone is subject to constant reconstruction throughout life with resorption and formation occurring simultaneously. During approximately the first two decades of life when growth takes place, the formation rate exceeds resorption leading to a net increase in bone mass. This period is referred to as the modeling phase, since in addition to the accrual of bone mass also substantial changes in the gross morphology of the bone occur. The morphologic changes include longitudinal growth of the long bones, which is achieved by bone formation at the endplates of the bones (epiphyseal growth plates), and radial growth due to bone formation on the outer surface of the cortex (periosteal apposition) and resorption on the inner surface (endosteal resorption).[8] Gradually the epiphyseal growth plates are closed and longitudinal growth and bone mass accrual is completed. After the modeling phase bone loss starts as a result of a decline in the formation rate in relation to the resorption activity. By this time the remodeling phase has started, characterized by a constant remodeling of the bone but largely sustaining the shape and size created during the modeling phase.[3,5] The underlying mechanisms for bone remodeling is found on the cellular level. There are basically three major cell-types in bone tissue. The osteoblast is a bone-forming cell located at the bone surface and has its origin from mesenchymal stem cells, which differentiate into osteoblasts after paracrine influence from growth and transcription factors. Once differentiated, this cell produces bone matrix (osteoid) constituted by primarily collagen type I and glycosaminoglycans. The matrix is thereafter subject to mineralization with crystalline calcium phosphate (hydroxyapatite), a process partly regulated by the osteoblasts by adjustment of the influx of mineral ions from the extracellular fluid. The osteoclast is the primary bone-resorbing cell, with its origin from hematopoetic stem cells. It is a large multinucleated cell which binds to the bone surface with adhesive proteins, creating a closed micro-environment where acidic hydrogen ions and proteolytic enzymes are secreted to resorb bone tissue. The osteocyte, originated from osteoblasts, is embedded within the bone with long dendrites spreading throughout the tissue. It has mechanoreceptors which sense mechanical loading on the bone and in response have regulatory effects on both osteoblasts and osteoclasts.[4,5,7]

Bone mass assessment

Bone mineral density – a surrogate of bone mass

Bone mass is often expressed in density, or rather, bone mineral density (BMD). The golden standard to measure BMD is by Dual-energy X-ray Absorptiometry (DXA).[9] As described later in the methods chapter, the given value is expressed as grams per square centimeter (g/cm^2), and is thus not a true volumetric density but rather an areal density (aBMD). Nonetheless, it is a robust method strongly associated to fracture risk, and used worldwide to evaluate bone health and diagnose osteoporosis.[10] An important issue is also that basically all pharmacological treatments indicated for impaired bone health today are developed and evaluated in relation to bone mass as measured by DXA. Since bone mass, and thereby BMD is very dependent on age, an arbitrary BMD measurement is difficult to interpret alone. For this reason BMD is usually standardized as a Z-score, and thus expressed as standard deviations (SD) below or over the mean in the respective age-group. To discriminate fragile bones from healthy bones in adults, the measured BMD is often expressed as SD below or over the mean BMD in young adult men and women respectively, and is then referred to as T-score.[9]

Geometry, volumetric density and microstructure

Although the DXA method is robust, accurate in reproducibility and strongly correlated to fracture risk, the geometrical structure and true volumetric density remains unrevealed with this method. To assess these properties in vivo, in a non-invasive manner, more detailed imaging techniques are needed. For macro-structural properties like cortical geometry and volumetric densities of both the cortical and trabecular compartment, quantitative computed tomography (QCT) can be used, allowing the examination of any chosen part of the skeleton.[11] A more commonly used method, both for clinical and research purposes, is the peripheral QCT (pQCT), which is able to scan the appendicular bones, e.g. lower leg or arm, rendering a single or multiple cross-sections of the region of interest. This method is often preferred due to a lower radiation dose, a lower price, and less inconvenience for the patient than whole body QCT. For higher resolution, enabling the assessment of cortical porosity and trabecular microstructure, high resolution QCT (HR-QCT) or high resolution magnetic resonance imaging (HR-MRI) can be used. These methods, used for bone research purpose only, can produce detailed three-dimensional images of chosen parts of the skeleton down to a resolution of less than one tenth of a millimeter.[6,12-14] Recently also biomechanical properties like failure load and stiffness can be estimated by

applying finite element analysis models on the image data from the HR-QCT device.[15,16]

Determinants of bone mass

BMD at any given point in adult life is determined by the maximum amount of attained bone mass, achieved during childhood and adolescence, peak bone mass (PBM), and the subsequent normal loss of bone with aging, for women especially due to the reduction of circulating estradiol following menopause.[17,18] Altogether, heritability is thought to account for about 60-80% of the age-specific variation in BMD in the general population.[19-21] The genes corresponding to this heritability are currently being mapped out by genome-wide association studies (GWAS), which has revolutionized the insight and understanding of several morbidities in man. Identified genes predicting bone mass are involved in biological pathways, regulating for example mesenchymal stem-cell differentiation (osteoblast/osteocyte), WNT-signalling (osteoblastogenesis) and RANK-RANKL-OPG pathway (osteoclast differentiation and activation).[22] Even though environmental factors answer for a smaller part of the variation in bone mass, they are still of great importance since they more easily can be targets for intervention. Well established environmental factors are nutrition (especially calcium and vitamin D intake), and lifestyle factors like alcohol intake, smoking and physical activity.[23,24] The mechanisms of the attainment of PBM are largely genetically determined but also environmental factors contribute.[19,25] The determinants of bone loss are, however, majorly of environmental origin while heritability in recent studies has been shown to account for about one fourth to one half of the between-individual variance of bone loss in both weight-bearing and non-weight-bearing bones in both men and women.[26-28]

Osteoporosis

Osteoporosis is a systemic degenerative skeletal disorder affecting men and women of increasing age worldwide.[29] It is characterized by low bone density and microarchitectural deterioration of bone tissue, and its main feature is an increased susceptibility for fragility, or low-trauma fractures.[9,30] The typical osteoporotic fracture sites include the distal forearm, lumbar and thoracic spine, the proximal humerus and the hip, with fracture incidence increasing steadily with age. Osteoporosis constitutes a major health concern worldwide in terms of both human suffering and financial cost. The age-adjusted incidence of fragility fractures has more than doubled since the 1950s in both men and women. According to estimates, the incidence will continue to

increase, partly as a consequence of an increased life expectancy.[31] As of today, the lifetime risk in Sweden at age 50, of having a fragility fracture is about 20% for men and 50% for women.[32] The criteria for the diagnosis of osteoporosis were established by the world health organisation (WHO) in 1994 for women, and defined as an aBMD of either the hip, spine or radius below -2.5 standard deviations (SD) of the mean in young adult women.[30,33] For men, almost twenty years later, no diagnostic criteria have been established, although the common practice is to use the same criteria as for women except with a young male reference population. Male osteoporosis, risk factors for fracture in men, and fracture preventive treatment in males are as of yet markedly understudied fields, although osteoporosis is a large health concern also in males.

Risk factors for osteoporotic fractures

One of the major risk factors for osteoporotic fractures is low BMD.[34] It has been shown that every SD decrease in BMD is associated with about a two-fold increase in the age-adjusted hip fracture risk in postmenopausal women, and with a three-fold risk increase in elderly men.[35-37] Other major risk factors are female sex, increasing age, previous osteoporotic fracture, family history of osteoporosis or fracture and systemic glucocorticoid treatment.[38-42] Weaker risk factors are low body weight, smoking, high alcohol consumption, low levels of vitamin D, hypogonadism, e.g. early menopaus in women, inactivity and all risk factors for falling, e.g. visual impairment and treatment with sedatives.[39] Recently the WHO introduced FRAX®, a web-based fracture risk assessment tool.[43,44] By using this tool it is possible to achieve an estimation of the 10-year risk of a major osteoporotic fracture, and hip fracture. The algorithm behind the tool is developed on data from prospective study cohorts all over the world, and by simply applying age, anthropometrics, prevalence of some of the aforementioned risk factors and aBMD of the femoral neck (optional), the risk is calculated in a population-specific manner, where the absolute fracture risk vary according to selected country.

Peak bone mass

Peak bone mass is the maximum amount of bone mass acquired during growth and sexual maturation.[45] PBM is generally claimed to be attained around the end of the second decade in life,[1] which is probably true regarding the most clinically relevant sites, such as the hip and spine.[46-50] This has been subject to debate over the last decades, and several studies, primarily of cross-sectional design but also prospective longitudinal studies, have reported

somewhat diverging results. For example, one cross-sectional study presented evidence indicating that the peak in hip bone mass had been reached already at twelve years of age in both sexes.[51] In contrast, there are longitudinal data reporting continuous increase in hip aBMD, as measured by DXA, up to thirty years of age in men.[52] As for the spine, one longitudinal study measuring the volumetric density by computed tomography, demonstrated that the peak in bone mass occur at the age of sexual maturity in females,[49] but there are also studies reporting continuous bone accrual or stable aBMD around the end of the third decade in life in both men and women.[46,53,54] Considering the limb-bones, e.g. the radius and tibia, the PBM is generally thought to be reached somewhere during the third or even the fourth decade of life. There are however lack of strong evidence, but a recent longitudinal study in men presented increments in both trabecular and cortical vBMD as well as an increasing cortical thickness due to a decrease of the endosteal circumference at the radius between 19-24 years of age.[50] The importance of the level of PBM for fracture risk at older age has been widely discussed over the past decades but naturally never proven.[17,18,55] PBM has been demonstrated to account for up to half of the variation in BMD at age 65, indicating an important role of the level of PBM on the risk of developing osteoporosis.[17,18,48] From a public health perspective it is thus of great importance to investigate the determinants of PBM, and reveal possible affectable factors to promote good bone health and thereby increase PBM in the population.

Hereditary determinants of peak bone mass

As previously mentioned, BMD at any point in life is primarily attributed to genetic factors and there are several studies showing that the majority of the genetic effect is most likely affecting the acquisition of PBM rather than bone loss.[25,56,57] The heritability of osteoporosis and fracture is rather well studied.[40] A number of studies have been performed on this subject, most of them indicating that both low BMD and previous fracture in a father, mother, or female sibling are associated with both low BMD and increased risk of fracture at various sites in both men and women.[23,36,40,58-62] Reports regarding hereditary influence around the time of PBM are however scarce. The significance of a fracture in a second-degree relative has not yet been elucidated, which could be of value when evaluating fracture risk in young persons, since their parents usually have not reached the age when fragility fractures occur. The role of the hereditary influence on traits, such as cortical bone size and volumetric BMD at the time of PBM, has also been less well studied. It is thus uncertain whether the pathway of the heritability consists of volumetric BMD (vBMD) or bone geometric properties.

Environmental determinants of peak bone mass

There are many environmental factors believed to contribute to the acquisition of PBM. Nutritional intake is one of them, where especially calcium and vitamin D are of importance. Calcium intake has been shown to increase bone mass accrual in randomized placebo-controlled trials (RCT) in both prepubertal girls and boys.[63,64] Vitamin D levels in the body are determined by dietary sources, by endogenous production in the skin when exposed to ultraviolet B radiation from the sun and by genetic factors.[65] Vitamin D has its primary effect on bone metabolism by increasing the intestinal absorption of calcium and phosphate, but it also has direct actions on osteoblast differentiation and regulation of osteoclast activity.[66] A finnish RCT reported a positive dose-response effect of vitamin D supplementation on bone mineral augmentation in adolescent girls with adequate calcium intake.[67] A recent meta-analysis of seven RCT:s suggested that supplemental vitamin D intake could increase bone mass in deficient children and adolescents.[68] There is, however, surprisingly little evidende that vitamin D increases PBM in subjects with normal vitamin D levels. Weight-bearing physical activity before and during puberty is also a significant contributor for an optimized PBM acquisition.[24,69,70]

Alcohol intake is a well known risk factor for osteoporotic fractures, even though most studies indicate that it takes a quite large amount equalling three or more units per day (1 unit = 285 ml of beer, 120 ml of wine, 30 ml of spirits) to consider it a contributor to fracture risk.[71,72] Regarding alcohol consumption and PBM acquisition there are no reports at this point.

Smoking is a major health-impairing lifestyle factor today, but evidence regarding the impact of smoking on skeletal health at young age is scarce. In middle-aged and the elderly there are rather convincing evidence that smoking negatively affects bone mass and is associated with increased fracture risk in both men and women.[73-76] As reported in a previous meta-analysis there also seems to be a remaining risk increase for fracture also in currently non-smoking men and women with a history of smoking.[74] In the context of PBM being a major determinant of fracture risk at old age, [17,18,77] this raises the question to what degree smoking affects bone mass during PBM acquisition. Previously, some small cross-sectional studies have been performed, demonstrating negative associations between smoking and areal BMD in both young men and women.[78-81] From baseline data of the Gothenburg Osteoporosis and Obesity Determinants (GOOD) cohort, it was suggested that smoking in 19 year old men opposed the normal age-dependent endosteal contraction (increase of the cortical thickness) of the

long bones, and had a negative effect on the trabecular bone at the tibia.[82] This was later essentially confirmed in another large study (n=677) on male siblings (25-45 years).[83] As of today there are no longitudinal studies investigating the impact of smoking in young adults around the time of PBM accrual.

A fracture history is one of the strongest risk factors for osteoporotic fractures.[84] Even though a prevalent fracture is not necessarily associated with low bone mass, it is well established that prevalent fractures in childhood and adolescence are associated with lower aBMD.[85-87] There are also studies reporting that childhood fractures are associated with low PBM acquisition, which in turn could be a predictor of persistent skeletal fragility, and the resulting low BMD in a young patient could thus be considered as a potential risk factor for future fracture.[88-90] Approximately one in three children suffer a fracture during growth,[91] with boys having a higher risk than girls of experiencing a fracture in the first 16 years of life.[92] Over the past 30 years, there has been a significant increase in distal forearm fractures in children and adolescents,[93] which in this context could indicate a decreasing PBM in the population with a consequent increased fracture burden in the future.[77] Thus, increased knowledge concerning bone mass in children may help to prevent fractures later in life.[94] Whether it is bone size or volumetric bone density that is associated with prevalent fractures in childhood and adolescence is not fully established, although a few cross-sectional studies on both men and women report that the strongest associations are found between fracture and trabecular volumetric density.[95-99] A couple of studies, so far, have also investigated how well trabecular microstructure, and finite element analysis derived biomechanical properties correlates to prevalent fractures at the age around PBM. These studies, one in adolescent boys and one in 20 year old women, reported that trabecular microstructure, failure load and stiffness were strongly associated to prevalent fractures.[95,96]

Novel factors

A recently published prospective study in a Brazilian cohort has shown a higher incidence of fracture between birth and age 11 in children of older mothers.[100] This could be a subject of concern for many populations and especially the Swedish population, since the maternal age in both primi- and multipara mothers has steadily increased during the last three decades. In this period, the mean age of mothers giving birth, both primi- and multipara included, increased from 26.0 to 30.3 years of age.[101] It has previously been reported that advancing maternal age increases the risk of fetal death,[102,103] but also of other morbidities in the offspring, such as chromosome

abnormalities, childhood cancers like leukemia and retinoblastoma, diabetes mellitus and schizophrenia.[104-107] It remains to be studied whether advancing maternal age have an influence on bone mass in the offspring.

AIM

The principal objective of this thesis was to identify, investigate and evaluate hereditary and environmental factors associated with the development of peak bone mass in Swedish men. Furthermore, an additional aim was to investigate which main features of the bone that was most associated with prevalent fractures in young adult men.

Specific aims

1. To examine whether prevalent hip fractures in second degree relatives (grandparents) was associated with lower aBMD and vBMD or with reduced bone size in young men at the approximate age of peak bone mass (Paper I).
2. To investigate if high maternal age was associated with the skeletal phenotype in the offspring at the approximate age of peak bone mass (Paper II).
3. To investigate the effect of changed smoking behavior on aBMD, vBMD and bone geometry in a 5-year longitudinal setting of 19 year old men (Paper III).
4. To investigate the possible associations between current smoking and parameters of trabecular microarchitecture of the radius and tibia in young men at the age of 24 years (Paper III).
5. To specifically determine which parameters of trabecular and cortical microarchitecture, geometry or measures of estimated bone strength of the bone that was most strongly associated with prevalent fractures from childhood to young adulthood in young adult men (Paper IV).

SUBJECTS AND METHODS

Subjects

All four papers included in this thesis were based on, or a subpopulation of, the Gothenburg Osteoporosis and Obesity Determinants (GOOD) study cohort. The GOOD study was initiated in 2003 with the aim to determine both environmental and genetic factors involved in the regulation of bone and fat mass. The cohort is well characterized, and consists of young adult men who were randomly identified through national population registers, and by telephone asked to participate in the study. For inclusion, subjects had to be between 18 and 20 years of age and willing to participate in the study. A total of 1068 young men with the mean age of 18.9 ± 0.6 (Mean ± SD) years at baseline were included, corresponding to 48.6% of the initially contacted study subjects. The cohort was found to be representative of the general young male population by comparing their anthropometrics with 624 aged-matched, randomly selected conscripts living in the same area as the GOOD-subjects. Using an independent samples t-test there were no significant differences in height, weight or BMI.[48] At the 5-year follow-up, 833 (78%) of the original subjects from the initial GOOD-study were included, after being contacted by letter and telephone. At follow-up, the mean age was 24.1 ± 0.6 years. To determine whether the cohort of the 5-year follow-up was representative of the original GOOD-cohort the variables of age, height, weight and physical activity at baseline were compared between the 833 included subjects and the 235 not included, using an independent samples t-test, where no significant differences were found.[50] A total of 128 (12%) declined to participate in the follow-up, and 107 (10%) could not be reached. The mean follow-up time was 61.2 ± 2.3 months, ranging from 55-70 months.

Paper I

The subjects in the first study, a study of cross-sectional design, consisted of the 1068 male subjects from the original GOOD study, with a mean age of 18.9 ± 0.6 years. Of these, 1015 could be linked to at least one grandparent in the multigeneration register, and were thus included in the statistical analyses.

Paper II

In the second study, a study of cross-sectional design, the full GOOD-cohort of 1068 male subjects with a mean age of 18.9 ± 0.6 years was included. Of

them we were able to identify the mothers to a total of 1009 GOOD men, who were included in the further analyses.

Paper III

The third study, which was a 5-year longitudinal study, included the 833 subjects enrolled in the 5-year follow-up in the GOOD-cohort. Their mean age at baseline was 18.9 ± 0.6 years, and at 5-year follow-up 24.1 ± 0.6 years.

Paper IV

The fourth study, of cross-sectional design, included 833 subjects, with a mean age of 24.1 ± 0.6 years, in the 5-year follow-up in the GOOD-cohort.

Ethical considerations and approvals

Written and oral consent was obtained from all study participants in the original GOOD-cohort, of which all, or subpopulations were included in paper I-IV. The studies were approved by the regional ethical review board at the University of Gothenburg, and the administrative authority Statistics Sweden approved the studies in paper I and II under the condition that grandparents, and mothers and fathers, respectively, were all de-identified. Hence, the authors could not distinguish any relative by name, social security number, or by any other means.

Questionnaire

Standardized self-administered questionnaires were used at both baseline and 5-year follow-up to obtain information about smoking (yes/no), calcium intake (mg/day), physical activity (hours/week) and fracture history. Regarding smoking, questions in the questionnaire were phrased: “How often do you smoke cigarettes?” and “Number of cigarettes/day?” Subjects claiming everyday smoking with at least one cigarette/day were regarded as smokers. Calcium intake was estimated from daily dairy product intake as mg/day, according to the Swedish National Food Agency’s Food Database, which provides information on the nutritional composition for more than 2000 foods and dishes. Subjects were asked to report the daily intake of milk or corresponding product (e.g. yoghurt or sour milk, 120 mg calcium/dl), number of slices of cheese (74 mg/slice) and whether they used any kind of multivitamin compound.[108] For amount of physical activity, subjects were asked about their present average amount of physical activity. Questions were

based on a validated physical activity questionnaire.[109] In all papers, physical activity was expressed as hours/week.

Register studies

Several registers administered by various Swedish authorities were used in paper I and II.

The Multi-generation register

This register, administered by the Swedish government agency Statistics Sweden, Population and Welfare Department, contains information of connections between all Swedish residents and their parents. The register was used to identify the second-degree relatives (grandparents) of the subjects in paper I, and the mothers and fathers of the subjects in paper II.

The Cause of Death Register

This register, administered by the National Board of Health and Welfare, contains information about the time and cause of death in persons registered in Sweden at the time of death. The register was used to establish if any of the grandparents in paper I, were deceased, and if so, at what time.

The National Patient Register

This register, operated by the National Board of Health and Welfare, includes information about all inpatient care occasions in the public health care system in Sweden, including diagnoses, codes of surgical procedures, dates, hospitals and clinics. From 2001 also all outpatient visits are registered, at both public and private caregivers. From this register we obtained information about hip fracture prevalence and type of hip fracture in the grandparents in paper I, by means of ICD codes. Only datable fracture were taken into account and were defined as fractures with codes of surgical procedures specified. This register was also used to verify reported fractures of the subjects in the GOOD cohort.

The Total Population Register

This register, administered by the Swedish government agency Statistics Sweden, Population and Welfare Department, includes information about migration (immigration and emigration). This information was used to specify the time of exposure to the Swedish health care system of the grandparents in paper I. That is, the period of time it would actually be

possible to find them in the patient register if they would have suffered a hip fracture.

The Medical Birth Register

This register, operated by the National Board of Health and Welfare, contains detailed information about practically all deliveries in Sweden since 1973, describing the medical circumstances at the time of childbirth. The register was used in paper II to provide information about maternal and offspring anthropometrics (height and weight), maternal age, smoking habits, parity, length of pregnancy and whether the delivery was ended vaginally or by caesarean section.

The National Archives – The Military Archives

The Military Archives, part of the National Archives, a Swedish government agency, were contacted to obtain the age of the fathers in paper II.

Swedish socioeconomic classification

Socioeconomic classification was retrieved as Socioeconomic index (SEI), from Statistics Sweden. SEI is a well-recognized classification based on the expected level of education that comes with a certain occupation. In paper II, this classification was obtained for the parents of the study subjects in the year of 1985 (subjects in the study were born between 1983 and 1985). Each study subject obtained a household SEI, which was determined by an order of dominance were the household received the highest SEI of the two parents.

X-ray verified fractures

In order to verify reported fractures from the questionnaire, various X-ray records in hospitals and clinics in the greater Gothenburg area were searched. By using the social security number of the study participants, we searched the centralized computerized X-ray registers including X-ray reports from 1991 in the three largest public hospitals in Gothenburg. Earlier X-ray reports were searched in a central archive, containing microfilmed reports from the entire Västra Götalandsregionen with a total of approximately 1.5 million inhabitants. To make the search as complete as possible we also searched the computerized archives of two private hospitals (Lundby and Carlanderska). As a last step, information from the national patient register was retrieved, as previously described. The X-ray reports were manually validated and subjects reporting a fracture that could not be verified were excluded from the analysis in paper III and IV. On the contrary, subjects who were found to

have a previous fracture without reporting this in the questionnaire were included as fractured subjects in paper III and IV.

Anthropometrics

Height and weight were assessed using standardized equipment. The Coefficients of Variation (CV) values were less than 1% at both baseline and the 5-year follow-up.

Bone measurements

Dual X-ray Absorptiometry (DXA)

The DXA technique is a widely used method, and the golden standard to assess bone mass in humans. It is based on X-ray technique, and therefore non-invasive. In short, the device scans the human body with two X-ray beams of different energy levels, allowing the separation of soft tissue and the more dense bone tissue. Sensors of the DXA will detect the absorbed amount of energy in different tissues of the body and produce an image of the mineralized bone and soft tissue in the chosen region of interest (figure 1). Since the amount of absorbed energy is dependent on density, a value of the density can be obtained, expressed as grams per square centimeter. Other measurements achieved are the scanned bone area and the bone mineral content of the scanned area. The DXA technique is thus a two-dimensional method unable to measure the true volumetric density. The effective radiation dose for a hip scan for an adult male is approximately 10 μSv, which corresponds to about 4 days of background radiation from e.g. the ground and space.[110,111]

Areal bone mineral density (aBMD; g/cm^2), bone mineral content (BMC; g) and bone area (cm^2) of the whole body, lumbar spine, total hip, femoral neck and nondominant total radius were assessed at both baseline and five-year follow-up in the GOOD-cohort, using the Lunar Prodigy DXA (GE Lunar Corp,. Madison, WI USA). The CVs for the aBMD measurements ranged from 0.5 to 3%. In paper II, all variables were used (aBMD, BMC and bone area), while only the aBMD measurements were used in paper I, III and IV.

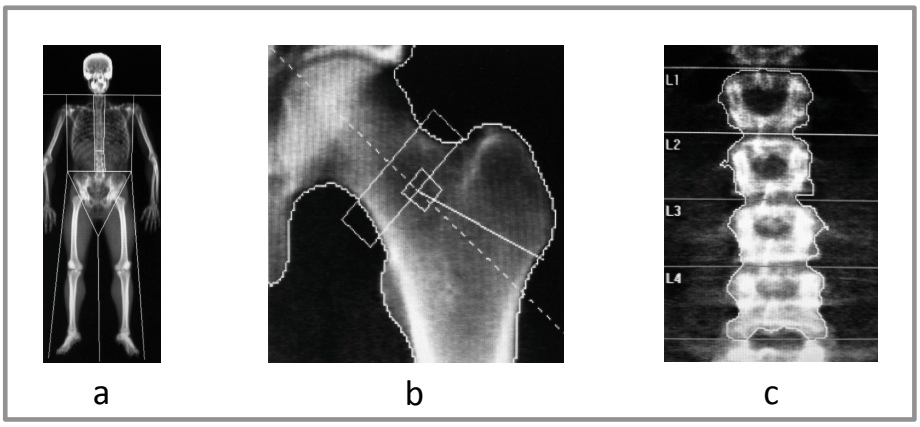

Figure 1. Images assessed by DXA (Lunar Prodigy, GE Lunar Corp., Madison, WI USA). a) Total body b) Total hip c) Lumbar spine

Periferal Quantitative Computed Tomography (pQCT)

A pQCT device contains a single X-ray source rotating around a fixed limb (lower leg or arm) of the body, producing an image of one or several cross-sections of the extremity. This allows a separation of cortical and trabecular bone, enabling investigation of both the geometrical properties, and volumetric density of the cortical and trabecular compartment respectively. The effective radiation dose is about 30 μSv per scan, and is restricted to the scanned cross-section (manufacturer specifications).

pQCT scans were performed at both baseline and five-year follow-up, using a single energy X-ray pQCT device (XCT-2000; Stratec Medizintechnik, GmbH, Pforzheim, Germany). Single scans were made on the non-dominant leg and arm through the diaphysis of the tibia and radius (at 25% of the bone length in the proximal direction of the distal end of the bone) to assess cortical parameters. The 25% site was chosen primarily because it is a diaphyseal site where there is almost exclusively cortical bone, and the site is readily accessible independent of the size of the study subjects. The obtained variables of the cortex were cortical volumetric bone mineral density (vBMD; mg/cm^3), cortical cross-sectional area (CSA; mm^2), cortical thickness (mm), endosteal and periosteal circumference (mm), and polar strength strain index of the cortex (SSI; mm^3). The SSI was calculated by the software, version 6.00 of the XCT-2000 and represents an estimation of the torsional resistance of the cortical bone.[112] Trabecular vBMD was measured using a scan through the metaphysis of the tibia and radius (at 4% of the bone

length in the proximal direction of the distal end of the bone), a site primarily composed of trabecular bone. The CVs were less than 1% for all pQCT measurements. All variables were used in paper I, II and III, except for SSI, which was only used in paper I.

High Resolution peripheral Quantitative Computed Tomography (HR-pQCT)

A HR-pQCT is a pQCT device with a higher resolution (82 μm in the present studies), performing several cross-sectional tomography slices in a row, producing a three-dimensional image of the bone (figure 2). This makes it possible to investigate microstructural properties of the trabecular bone, such as amount of trabeculae, trabecular thickness and separation. The effective radiation dose from one scan is approximately 5 μSv and is restricted to the scanned region (manufacturer specifications).

A high-resolution 3D peripheral quantitative computed tomography (HR-pQCT) device (XtremeCT; Scanco Medical AG, Brüttisellen, Switzerland) was used at the five-year follow-up of the GOOD study, to scan the ultradistal tibia and radius of the non-dominant leg and arm, respectively. The procedure of measuring the volume of interest (VOI) was executed according to a standardized protocol as described elsewhere.[12,113] Briefly, a reference line was manually placed at the centre of the scan of the end plate of the distal radius and tibia. The first computed tomography slice started 9.5 and 22.5 mm proximal to the reference line for the radius and tibia, respectively. A total of 110 parallel computed tomography slices, with a nominal isotropic resolution (voxel size) of 82 μm, were obtained at each skeletal site, delivering a 3D representation of an approximately 9-mm section of both the radius and tibia in the proximal direction. All image analysis was performed in a custom-built Image Processing Language (IPL Version 5.06a-ucsf, Scanco Medical AG), and according to this previously described method,[12] we obtained cortical thickness (μm), cortical cross-sectional area (CSA, mm^2), cortical volumetric BMD (vBMD, mg/cm^3), trabecular bone volume fraction (BV/TV, %), trabecular number (mm^{-1}), trabecular thickness (μm), and trabecular separation (μm). The CVs ranged from 0.3% to 3.9% for the radius, and from 0.1% to 1.6% for the tibia. The same device, software and operator were used throughout the study. All measurements were evaluated due to a five-item graded scale as recommended by the manufacturer (Scanco Medical AG), where 1 corresponded to highest quality, 2 to 3 to acceptable quality, and 4 to 5 to unacceptable quality. Measurements graded from 4 to 5 were excluded from the analysis. Only the trabecular variables were used in paper III, whereas all variables were used in paper IV.

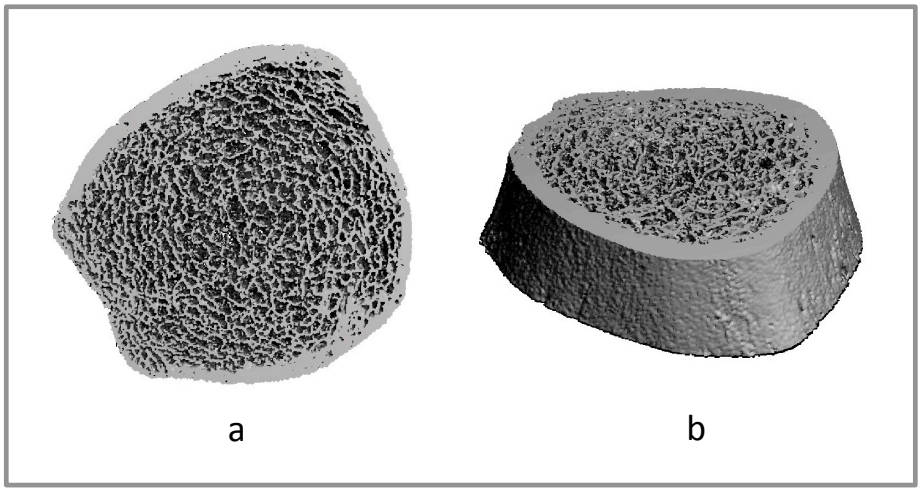

Figure 2. Images assessed by HR-pQCT (XtremeCT; Scanco Medical AG, Brüttisellen, Switzerland). a) Cross-section of the ultradistal tibia. b) 3D-image of a 9-mm section of the ultradistal tibia.

Ultra-Distal Cortical Evaluation

From the image data assessed with the HR-pQCT, the cortical microstructure is also obtainable by performing ultra-distal cortical evaluation. This is done by using the Cortical Autocontouring and Eval Crtx 6x softwares, incorporated in the manufacturer's Image Processing Language (IPL) software (μCT Evaluation Program v6, Scanco Medical AG, Brüttisellen, Switzerland).[6,14] In summary, endosteal and periosteal contours are automatically created to distinguish the boundaries of the cortical compartment in the VOI, excluding trabecular bone and extra-osseal soft tissue, respectively. Thereafter all void voxels within the cortical compartment are identified, and by further processing, the Haversian canals are distinguished from artefacts due to surface roughness and transcortical foramen or erosions. Finally these images are digitally superimposed, generating a refined cortical compartment region in the VOI (figure 3).[114] By using this method we obtained cortical porosity (%) and mean cortical pore diameter (μm). The CVs for porosity were 15.9% at the radius and 5.5% at the tibia, and the CVs for mean cortical pore diameter were 6.0% at the radius and 3.9% at the tibia. These variables were used in paper IV.

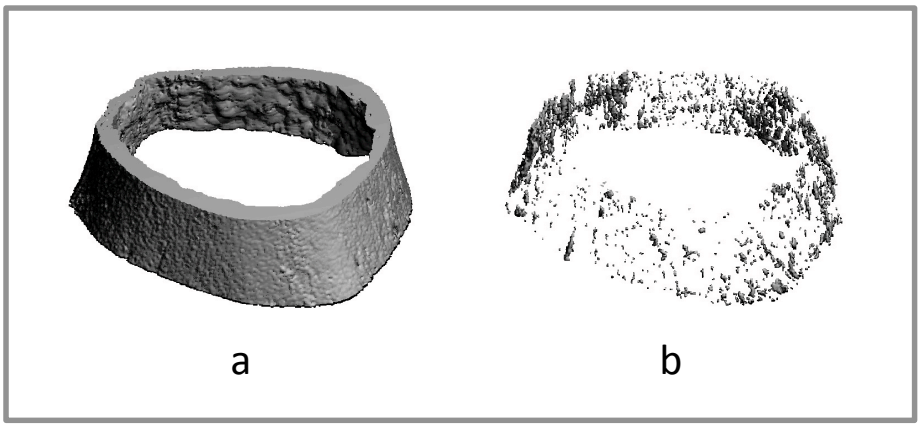

Figure 3. Images assessed by HR-pQCT (XtremeCT; Scanco Medical AG, Brüttisellen, Switzerland). a) 3d-image of the cortex at the ultradistal tibia b) 3D-image of the cortical porosity at the ultradistal tibia

Finite Element Analysis

For an estimation of the biomechanical properties of the bone, Finite Element Analysis (FEA) can be applied on the image data retrieved by the HR-pQCT. This is performed by the Finite Element software (version V5.11/FE_V01.15), provided by the manufacturer (Scanco). To summarize, cortical and trabecular bone are first separated by a script provided in the software. Micro FE models are then created by converting each voxel in the model to an equally sized brick element.[115] Material properties are then chosen as isotropic and elastic. In the model used in the present project an elasticity of a Young's modulus of 10 GPa was assigned to both cortical and trabecular elements, and a Poisson ratio of 0.3 was used for all elements.[15] As a next step, a simulated compression is applied in the longitudinal direction of the bone, at the radius corresponding to a fall from standing on an outstretched hand, representing the type of trauma involved in Colles fracture.[116] Using this method, we assessed stiffness (kN/mm), failure load (N), percentage of load carried by the trabecular bone at the distal and proximal surface of the VOI, respectively (percent load trabecular distal, and percent load trabecular proximal, respectively). The CVs ranged from 0.8% to 3.9% at the radius and from 0.2% to 3.0% at the tibia for these measurements. These variables were used in paper IV.

Statistics

All statistical analyses were performed using the SPSS software (SPSS Inc., Chicago, IL, USA)(Paper I-II; version 16.0, Paper III; version 17.0, and paper IV; version 20.0).

For comparisons of the means of bone variables, environmental factors and anthropometrics between groups in paper I, II and IV, independent samples t-test was used. In paper III, ANOVA, and post hoc least significant difference test was used to analyze differences of the means, and means of longitudinal changes, between groups. For categorical variables chi square test was used in all papers. At both baseline and 5-year follow-up of the GOOD-cohort weight was not normally distributed and therefore log transformed. In paper I, II and III, means of bone variables were adjusted for age, smoking, calcium intake, physical activity, height and weight using linear regression equations. For calculations of hereditary scores in paper I, a hazard function was used, estimated with a Poisson model,[117] and based on hip fracture in register, age at start of register, time in register, and sex of grandparent. The association between hereditary score and hip fracture in a grandparent were investigated using multivariate linear regression analysis. In paper II, Pearson correlation was used to investigate bivariate correlations between both maternal age and aBMD of the lumbar spine, and all other presented characteristics of the GOOD-cohort and their parents. The independent predictors of bone parameters in the same paper were assessed using a stepwise linear regression model. For presentation of the graphs illustrating the adjusted association between maternal age and lumbar spine aBMD in paper II, multiple regression using spline functions was applied. In paper IV, associations between fracture prevalence and bone measurements was investigated using a logistic regression model, including age, smoking, calcium intake, physical activity, height and weight as covariates. For predictors of failure load in paper IV, stepwise linear regression equations were used, where R^2 and R^2 change were calculated to evaluate the role of each independent variable. For all analyses a value of $p < 0.05$ were considered significant.

RESULTS

Paper I

Hip fracture prevalence in grandfathers is associated with reduced cortical cross-sectional bone area in their young adult grandsons

The objective of this cross-sectional study was to examine whether hip fracture prevalence in grandparents was associated with lower areal and volumetric BMD or with reduced cortical bone size in a large cohort of 1068 young adult men. A total of 3688 grandparents of 1015 grandsons (18.9 ± 0.6 years) (mean ± SD) were identified, and included in the analyses.

Results

- Grandsons of grandparents with hip fracture (n = 269) had lower aBMD at the total body, non-dominant radius, and lumbar spine, but not at the hip, than grandsons of grandparents without hip fracture.

- Grandsons of grandparents with hip fracture had reduced cortical cross-sectional area at the radius as compared to grandsons of grandparents without hip fracture.

- Subgroup analysis demonstrated that grandsons of grandfathers with hip fracture (n = 99) had substantially lower aBMD at the total body (2.9%, $p = 0.001$), lumbar spine (4.9%, $p < 0.001$) and total femur (4.1%, $p = 0.003$), and lower cortical cross-sectional area of the radius (4.1%, $p < 0.001$) and tibia (3.3%, $p = 0.01$).

- The polar strength strain index, a parameter of bone strength, was considerably lower in the radius in grandsons of grandfathers with hip fracture (6.8%, $p < 0.001$).

- Adjusting bone variables for grandson age, weight, height, smoking, calcium intake, and physical activity, and taking grandparent age at register entry, years in register, and grandparent sex into account strengthened or did not affect these associations.

Conclusions

In conclusion, family history of a grandfather with hip fracture was associated with reduced aBMD and cortical bone size in 19-yr-old men,

indicating that patient history of hip fracture in a grandfather could be of value when evaluating the risk of low bone mass in men.

Paper II

Advancing maternal age is associated with lower bone mineral density in young adult male offspring

The aim of this cross-sectional study was to investigate whether a high maternal age was associated with lower peak bone mass, as measured using DXA and pQCT, in a large cohort of 1068 young adult male offspring. The mothers were identified in a total of 1009 subjects (18.9 ± 0.6 years), who were included in the further analyses.

Results

- Maternal age at childbirth was inversely correlated to areal BMD of the total body, lumbar spine and non-dominant radius.

- Maternal age was found to be a negative predictor of areal BMD at the lumbar spine ($\beta = -0.09$, $p < 0.01$), independently of variables correlated to lumbar spine aBMD, and socioeconomic status, maternal smoking, parity and anthropometrics, and paternal age.

- Increasing maternal age was also independently associated with a reduced bone area of the lumbar spine ($\beta = -0.06$, $p < 0.05$), and a smaller cortical bone size of the radius (periosteal ($\beta = -0.16$, $p < 0.001$) and endosteal ($\beta = -0.19$, $p < 0.001$) circumference), but not with trabecular or cortical vBMD of the radius, in the offspring.

- Mothers > 36 years (90^{th} percentile) had sons with lower areal BMD at the total body (1.6%, $p = 0.005$), lumbar spine (2.6%, $p = 0.02$) and femoral neck (2.8%, $p = 0.01$), and cortical cross-sectional area of the radius (2.0%, $p < 0.05$).

Conclusions

In conclusion, the results suggest that advancing maternal age could negatively affect bone mass in young adult men, which could, at least partly, be due to a smaller bone size.

Paper III

Smoking is associated with impaired bone mass development in young men: A five year longitudinal study

The primary aim of the study was to test the hypothesis that smoking impairs bone-development, by investigating the development of areal and volumetric BMD, and bone geometry in a five-year longitudinal study of 833 young adult men (18.9 ± 0.6 years) (baseline). The secondary aim was to perform a cross-sectional analysis, investigating the possible associations between current smoking and parameters of trabecular microarchitecture of the radius and tibia, in young men (24.1 ± 0.6 years) (5-year follow-up).

Results

- Subjects who had started to smoke since baseline had about one-half as much increase in aBMD at the total body and lumbar spine, and about twice as much decrease in aBMD at the total hip and femoral neck at 5-year follow-up, as subjects who where nonsmokers at both baseline and follow-up.

- At the tibia, subjects who had started to smoke had a smaller increment of the cortical CSA than nonsmokers (8.1 ± 4.3 mm^2 vs. 11.5 ± 8.9 mm^2, $p = 0.03$), and a considerably larger decrement of trabecular vBMD than nonsmokers (-13.9 ± 20.5 mg/mm^3 vs. -4.1 ± 13.9 mg/mm^3, $p < 0.001$).

- Men who had continued to smoke since baseline had lower trabecular bone volume fraction at the tibia than nonsmokers (17.3 ± 2.7 % vs. 18.4 ± 2.7 %, $p = 0.03$), which was due to a lower trabecular thickness (8.9%, $p < 0.001$).

Conclusions

In conclusion, men who start to smoke in young adulthood have poorer developement of their aBMD than nonsmokers, possibly due to a lower trabecular density and smaller cortical cross-sectional area. Our results indicate that smoking around the time of peak bone mass could affect the trabecular bone primarily by means of thinning the trabeculae.

Paper IV

X-ray verified fractures are associated with finite element analysis derived bone strength and trabecular microstructure in young adult men

The aim of this cross-sectional study was to investigate whether a prevalent fracture was related to impaired trabecular and cortical microstructure and finite element analysis estimated bone strength in 833 young adult men around the approximate time of peak bone mass (24.1 ± 0.6 years).

Results

- A total of 292 study subjects had experienced at least one prevalent fracture, while 468 had no previous fractures.

- Men with prevalent fractures had lower areal BMD of the total body and non-dominant radius, and lower trabecular bone volume fraction (5.5%, $p < 0.001$), as a result of lower trabecular thickness and higher trabecular separation at both the radius and tibia. At the tibia, but not the radius, also the cortical thickness (5.1%, $p < 0.01$) and cross-sectional area (4.1%, $p < 0.01$) was lower.

- Using a logistic regression model (with age, smoking, physical activity, calcium intake, height and weight as covariates), BV/TV was inversely and independently associated with prevalent fractures (OR 1.28 (1.04-1.59)), whereas aBMD and cortical thickness was not (OR 1.19 (0.92-1.55) and OR 0.91 (0.73-1.12), respectively).

- No significant associations were found between prevalent fractures and the cortical microstructural parameters of porosity or mean pore diameter at either the radius or tibia.

- FEA estimated bone strength failure load, was inversely associated with prevalent fractures at both the radius (OR 1.22 (1.03-1.45)) and tibia (OR 1.32 (1.11-1.56)). The trabecular bone at the distal end of the radius carried a smaller fraction of the load in men with prevalent fractures than in men without fractures.

- The prevalence of a childhood fracture (≤ 16 years) was inversely, and more strongly than any prevalent fracture, associated with trabecular microstructure, cortical geometry and estimated bone strength parameters of both the radius and tibia.

Conclusions

In conclusion, prevalent fractures in young adult men were associated with low trabecular microstructure at the radius, independently of aBMD and cortical thickness, indicating that primarily trabecular bone deficits are of importance for prevalent fracture in this population. Fractures occurring in childhood, before the age of 17 years, seem to be the strongest contributor to this association.

DISCUSSION

The purpose of the present thesis was to investigate factors related to peak bone mass acquisition in men, in order to contribute to a better understanding of the variation of bone mass in the male population, which, so far, has been understudied. The significance of a maximized PBM at young age in relation to osteoporotic fractures later in life has not yet been fully investigated. It is, however, reasonable and generally accepted that a higher PBM in the population would postpone the effect of the natural bone loss occurring in both men and women, and consequently perhaps also lower the incidence of osteoporotic fractures in the future.[77] Increased knowledge about the timing and determinants of PBM is therefore essential in aspects of both early identification of individuals at risk for impaired skeletal health, but also for the discovery of treatment targets and development of early interventional methods to optimize PBM.

In this thesis, four potential determinants of PBM of different nature were investigated in a cohort of 1068 young adult men between 18 to 20 years of age at baseline (the GOOD-cohort), namely heredity in terms of fracture in second-degree relatives, and the other factors including advancing maternal age, smoking and fracture history. The key findings are visually presented in figure 4 and 5.

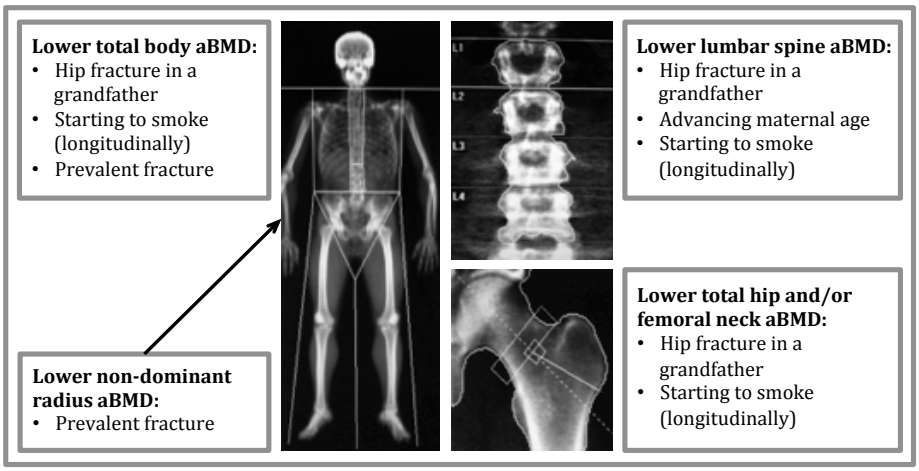

Figure 4. Images of the total body, lumbar spine and total hip as assessed by DXA (Lunar Prodigy, GE Lunar Corp., Madison, WI USA). Key findings of the thesis.

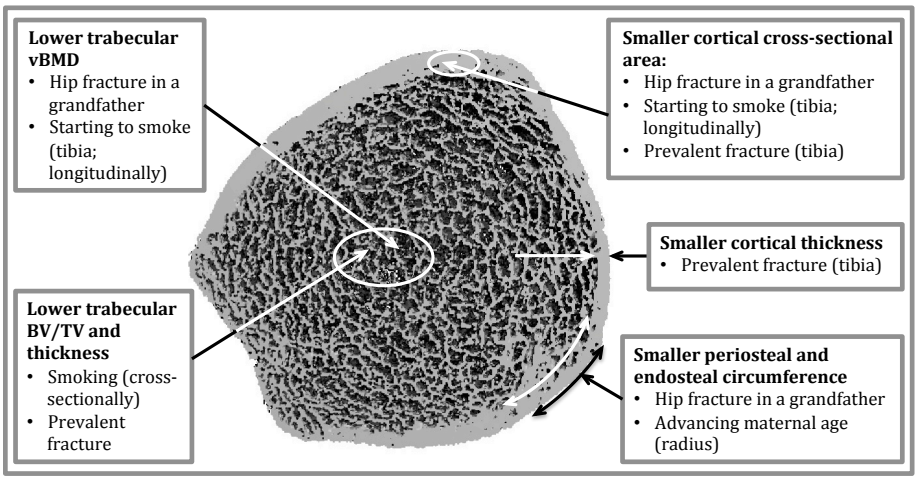

Figure 5. Cross-section of a distal tibia, representing a tubular limb-bone, assessed by HR-pQCT (XtremeCT; Scanco Medical AG, Brüttisellen, Switzerland). Key findings of the thesis.

Paper I

Hip fracture in a grandparent

In the first paper we reported that a hip fracture in a grandparent was associated with lower aBMD at several sites, and reduced cortical cross-sectional area at the radius in 18-20 year old men. We also demonstrated that the estimated bone strength as reflected by strength strain index, which is dependent on the cortical size, was lower at the radius in men with a second degree relative with a prevalent hip fracture. Several studies have reported about heredity for bone mass, fracture and osteoporosis over one generation,[23,36,40,58-62] but this is the first study reporting of a possible hereditary trait over two generations. This is a reasonable finding since hip fracture in particular has been shown to be a strong indicator of a hereditary component, used for example in the web-based fracture risk assessment tool FRAX®.[43] It is also reasonable that this is traceable already around the time of PBM, since the heredity for bone mass have been demonstrated to affect the acquisition of PBM to a higher extent than bone loss.[25] What is surprising though, is that in the studied population, the hereditary trait seems to be driven by hip fractures in a grandfather rather than grandmother. Further subanalysis, not published in the paper, also indicated that it was the maternal

grandfathers who were responsible for the majority of the difference. This subanalysis had however severe problems concerning the statistical power in the study, since the number of maternal grandfathers was rather small (n = 50). The overall benefit of this finding is that it could be of value when evaluating the heredity for fracture risk in young persons, since their parents usually have not reached the age when fragility fractures occur.

Paper II

Advancing maternal age

In the second paper, we reported on the impact of late childbearing on peak bone mass, from the male offspring's point of view. Our results revealed significant associations between an increased maternal age, and both bone mineral density and bone mineral content. We also reported that the DXA derived projected bone area was inversely associated with maternal age, which could indicate that the association was dependent on a bone size difference. When investigating the radius by means of pQCT, both the endosteal and periosteal circumference was inversely associated with maternal age, whereas the volumetric density was not, indicating that the lower aBMD was, at least partly, caused by a smaller bone size at the radius. Several possible explanations for this association is discussed in paper II, such as height of the mother, socioeconomic status, and other offspring characteristics known to affect bone mass acquisition. Adjusting for these available predictors did however not explain the found association between bone mass and maternal age. Plausible, though not possible to adjust for in the present study, are epigenetic causes.

Late childbearing is associated with increased risk for both fetal death and morbidity in the offspring.[102,106,107] In Sweden, maternal healthcare prenatal diagnosis is offered to pregnant women older than 35 years of age, mostly due to the fact that this age is a cut-point for the risk of chromosomal abnormalities in the offspring.[106] The negative impact of advancing maternal age on bone mass reported in paper I is not nearly of such magnitude that it should be considered a risk for impaired skeletal health in the individual offspring. It may however be of importance on a population level. Reports of an increasing age in mothers giving birth to their first child put this to perspective,[101] since a shift in maternal age of several years in a population could shift the distribution of bone mass, and thereby cause several additional individuals to be diagnosed with osteoporosis, and thereby increase the overall fracture incidence in the population in the future.

In 2009 a prospective study in a Brazilian cohort showed that the mother's age at the time of childbirth was positively associated with an increased fracture incidence in their children, from birth to early adolescence.[100] Unfortunately we were not able to investigate whether maternal age was associated with prevalent or incident fractures in the GOOD-cohort due to lack of ethical permission. We suggest that large epidemiological studies investigating the relationship between maternal age, peak bone mass and fracture risk are needed.

Paper III

Smoking

Smoking is associated with impaired health in general, and in paper III we longitudinally investigated the impact of altered smoking habits on bone development around the time of peak bone mass. We demonstrated substantial differences between nonsmokers and subjects who started to smoke between 19 and 24 years of age, where the latter subjects had about half the increase in aBMD of the total body and the lumbar spine as compared to the nonsmokers. In the hip region, where aBMD was decreasing in the study population, the decrement was about twice as high in subjects who started to smoke. According to our results, this was likely primarily due to an impaired development of the trabecular bone, but likely also due to a poor development of cortical cross-sectional area. This is the first longitudinal study investigating the effect of smoking on bone mass as assessed by both DXA and pQCT in the young. Two cross-sectional studies on young men have, however, also reported of primarily trabecular deficits in smokers,[82,83] and when performing a cross-sectional analysis at 5-year follow-up in the GOOD cohort we found that HR-pQCT derived trabecular bone volume fraction at the tibia was considerably lower in smokers than in nonsmokers, which was likely the result of thinner trabeculae. The rather large possible impact of smoking on bone mass in young adulthood demonstrated in paper III, encourages the important task of promoting a nonsmoking lifestyle in young males.

Paper IV

Fracture history

In the final paper we emphasize the prevalence of a fracture as a possible risk factor for suboptimal peak bone mass acquisition. The association between prevalent fractures and aBMD in adolescence and young adulthood have been subject to a few previous studies in men, of which practically all have

indicated that trabecular vBMD is of importance.[95,97,99] In paper IV we report in the largest study so far performed, that men with prevalent fractures had reduced bone volume fraction primarily due to thinner trabeculae with a higher separation. Our data also suggest that the association is primarily driven by fractures occuring in childhood, that is, before the age of 17 years. Applying finite element analysis models on the HR-pQCT data, also estimated bone strength parameters appears to be associated with prevalent fracture. This strengthens the hypothesis that a prevalent fracture, not only in the elderly, but also in the young, could be considered a warning signal of a potentially impaired adult bone strength. Furthermore, we suggest that measuring trabecular microstructure could be superior to DXA when evaluating bone health at the time of peak bone mass.

Magnitude of identified associations

To better grasp the associations found between bone mass and some of the factors presented in the thesis, a comparison with physical activity and its association with bone variables could be attempted. Physical activity is a well studied determinant of peak bone mass, and considered a predictor of bone mass and especially cortical bone size.[118,119] Physical activity has previously also been thoroughly studied in the GOOD-cohort. Nilsson et al, demonstrated e.g. in a cross-sectional study that currently inactive GOOD-subjects who had been physically active during growth had a 6.9% larger cortical cross-sectional area and 3.2% wider periosteal circumference at the tibia, than subjects who had always been inactive.[120] The heredity factor of having a grandfather with a hip fracture, described in paper I, was also primarily related to the cortical bone size, and the difference in cortical cross-sectional area and periosteal circumference at the radius were 4.1% and 2.5%, respectively, as compared with subjects without a grandfather with hip fracture. Maternal age >36 years of age was also related to the cortical bone size in the offspring, as demonstrated in paper II. The differences were in the magnitude of 2.0% lower cortical cross-sectional area at the radius as compared to the offspring of mothers 36 years and younger. In paper IV, subjects with a prevalent fracture had 4.1% lower cortical CSA at the tibia than subjects without prevalent fractures. To sum up, the magnitude of the associations between bone size and hip fracture in a grandfather and a prevalent fracture, respectively, are comparable to the magnitude of the reported differences in cortical bone size between men who were physically active during bone mass accrual and men who were sedentary, while

maternal age displayed somewhat smaller possible magnitude of the association.

Nilsson et al also reported, in a 5-year longitudinal study from 19 to 24 years of age in the GOOD cohort, that subjects who had increased their physical activity from low (< 4 hours/week) to high (≥ 4 hours/week) had 7.1% gain of lumbar spine aBMD, whereas men with a consistently low physical activity gained 3.7%. At the tibia the corresponding 5-year changes of trabecular vBMD was 0.6% and 2.1% decrease, respectively.[121] The nonsmokers in paper III increased 4.2% in lumbar spine aBMD over the same period, whereas subjects who started to smoke only increased 2.2%. At the tibia, nonsmokers lost 1.5% in trabecular vBMD while those who started to smoke lost 5.3%. This suggests that a, most likely, positive effect on bone of an increased physical activity from 19 to 24 years of age, could theoretically be reversed by starting to smoke over the same period.

More importantly, there is also a posibility that the investigated determinants in the thesis may be independent and have additive effects on bone mass acquisition. Thereby, the studied determinants could have a quite substantial impact on peak bone mass in for example a smoking young man with a mother giving birth at age 35, a prior fracture, and a grandfather with a hip fracture.

Covariation of the bone measurements

In basically all papers in the thesis, a quite large number of variables in each subject were statistically tested in different manners, and additional sub-analyses were performed. The issue of correcting for multiple testing thereby deserves some attention. That is, with a significance level of 0.05, one out of 20 tests will, by chance, be significant. In defense of the quite comprehensive number of analyses included in the thesis, several of the bone measurements are closely related. That is, many of the variables are surrogates of the same measure. To exemplify this covariation, correlation coefficients (r) between some of the frequently used variables in the thesis are presented in table 1 and 2. Several of these correlations have previously been presented, although in a sub-sample of the GOOD-cohort.[122] Furthermore, the significant variables in the various studies were, almost without exception, part of a pattern of several other significant findings in the same category, indicating that the findings also were reasonable. Low p-values for the key findings, e.g.

cortical bone size in paper I, lumbar spine aBMD in paper II, trabecular vBMD and BV/TV in paper III and IV, respectively, also strengthens the hypothesis that the found associations was not the result of chance.

Table 1. Correlations between areal BMD measurements at baseline (18-20 years) in the 1068 young adult men of the GOOD cohort

	Total body	Lumbar spine	Total hip	Femoral neck	Radius
Total body	1	0.81	0.85	0.82	0.66
Lumbar spine	0.81	1	0.74	0.70	0.49
Total hip	0.85	0.74	1	0.93	0.55
Femoral neck	0.82	0.70	0.93	1	0.56
Radius	0.66	0.49	0.55	0.56	1

r-coefficients are presented. Bivariate Pearsson correlation were used.
All correlations were significant to a level of $p < 0.001$

Table 2. Correlations between measurements of cortical bone size at baseline (18-20 years) in the 1068 young adult men of the GOOD cohort

	Radius				Tibia			
	CSA	TH	PC	EC	CSA	TH	PC	EC
Radius								
CSA	1	0.71	0.84	0.42	0.58	0.36	0.52	0.25
TH	0.71	1	0.21	-0.35	0.41	0.48	0.11	-0.19
PC	0.84	0.21	1	0.84	0.49	0.14	0.64	0.49
EC	0.42	-0.35	0.84	1	0.25	-0.14	0.55	0.57
Tibia								
CSA	0.58	0.41	0.49	0.25	1	0.79	0.71	0.17
TH	0.36	0.48	0.14	-0.14	0.79	1	0.13	-0.47
PC	0.52	0.11	0.64	0.55	0.71	0.13	1	0.81
EC	0.25	-0.19	0.49	0.57	0.17	-0.47	0.81	1

r-coefficients are presented. Bivariate Pearsson correlation were used.
All correlations were significant to a level of $p < 0.001$
CSA - cortical cross-sectional area, TH - cortical thickness, PC - cortical periosteal circumference, EC - cortical endosteal circumference

Peak bone mass in the GOOD-cohort

The age of attainment of PBM is site-specific, but generally PBM is defined as a period of stable BMD levels after bone mass accrual and growth, prior to age-related bone loss. The reigning consensus is that PBM is achieved around the end of the second decade in life.[1] The assumption that the GOOD-cohort, used in the present thesis, is at the age of PBM could however be a source of dispute. In 2005, cross-sectional data of the GOOD-cohort was published on this subject by Lorentzon et al, where evidence that PBM had been reached at

the lumbar spine, femoral neck and total body, were presented. There is however a risk of both under and overestimating the rates of both gain and loss in bone mass with a cross-sectional design,[123] and longitudinal studies in general are associated with a higher degree of evidence. Recently, Ohlsson and Darelid et al published 5-year longitudinal data of the GOOD-cohort with bone measurements derived by both DXA and pQCT at baseline and 5-year follow-up in a total of 833 subjects.[50]

Hip region

Ohlsson and Darelid reported that the aBMD of the total hip and femoral neck in the GOOD cohort was decreasing with 1.9% and 3.6% respectively, between 19 and 24 years of age, indicating that the PBM had already been reached at this site.[50] In paper III, we reported that the magnitude of the decrease in the hip region between 19 to 24 years of age in the GOOD-cohort was more or less doubled in subjects starting to smoke (n=31) compared to the vast majority of nonsmokers (n=736), indicating an approximately doubling of the normal rate of bone loss in men starting to smoke in their early twenties. This also suggests that the reported negative impact of starting to smoke on hip aBMD could be the result of enhanced bone loss rather than impaired peak bone mass acquisition. From the results in paper III it was however also observed that subjects smoking at baseline had lower aBMD already from study start indicating that there, in addition, is an effect of smoking on PBM acquisition. This has previously been demonstrated in a cross-sectional study on the original GOOD cohort by Lorentzon et al.[82] In paper I, GOOD subjects with a grandfather with hip fracture had about 4% lower aBMD at both the total hip and the femoral neck at 19 years of age, than subjects without a grandfather with hip fracture. Although different study-designs, the difference of the means approximately corresponds to the magnitude of the normal 5-year loss at the femoral neck, whereas it exceeds the loss at the total hip with more than double. Furthermore, according to the 5-year longitudinal follow-up study of the GOOD cohort,[50] it is reasonable to presume that the study in paper I actually was performed at, or around the age (19 years) of peak bone mass regarding the hip region. Our results thus imply that heritability for hip fracture is, at least partly, mediated by PBM accrual. The statement that the GOOD cohort has passed the plateau of PBM at the hip by 23-25 years of age is also well in line with reports from several other longitudinal studies investigating PBM in young men. Bachrach et al,[47], reported peak aBMD of the total hip to be present at 16 years of age. Berger et al,[46] demonstrated the peak in total hip aBMD to occur between 19-21 years of age, and finally, Jackowski et al,[124] reported that the PBM of the hip region occured between 19-22 years of age depending on the site in the hip (femoral neck, intertrochanteric and femoral shaft).

Lumbar spine

Regarding the lumbar spine in the GOOD cohort, Ohlsson and Darelid reported an increase in aBMD of 4.2% between 19 and 24 years of age.[50] This is fairly in agreement with a longitudinal study by Berger et al,[46] who demonstrated the peak in DXA derived lumbar spine aBMD to occur somewhere between 19 and 33 years of age. There are however two longitudinal studies by Bachrach et al and Boot et al, reporting peak aBMD at 16 and 21 years of age, respectively,[47,125] and despite a significant 5-year increase in the study on the GOOD cohort, the authors do not strictly draw the conclusion that bone acquisition is still ongoing.[50] Evidence have been published by Wren et al, showing that QCT derived vBMD of the spine was reached at sexual maturity in females, while DXA derived aBMD continued its increase. The authors suggested that this was due to changes in surrounding soft tissue rather than changes within the vertebral body.[49] Ohlsson and Darelid et al hypothesize that the longitudinal 5-year gains of lumbar spine aBMD observed in the GOOD-cohort could partly be due to an increase of abdominal fat, since the weight of the subjects had increased by approximately 5 kg between baseline and follow-up.[50] This raises the question to what extent a DXA derived lumbar spine aBMD is confounded by fat mass and weight. Of the bone parameters in paper II, we reported that lumbar spine aBMD at age 19, was most strongly associated with maternal age. Maternal age in turn, was of borderline significance associated with weight, but also of borderline significance inversely associated with total body fat mass, indicating that this could be a confounder. Adjusting for both weight and total body fat mass in the statistical analyses in paper II did however not affect the association between advancing maternal age and low lumbar spine aBMD. Thus, it is unlikely that either weight or fat mass would have had an influence on the found associations. As for the PBM of the lumbar spine, there are thus some evidence pointing towards a peak in aBMD around the end of the second decade in life, but also evidence for a continous bone mass accrual in the third decade of life, and further QCT studies of longitudinal designs are needed to fully elucidate this matter.

Appendicular sites

Concerning the appendicular skeleton, the aBMD of the radius increased 7.8% in the GOOD cohort between 19 and 24 years of age. This was partly due to an increased vBMD of both the cortical (2.1%) and trabecular (2.9%) bone. At the tibia, not evaluated by DXA, the cortical vBMD increased 0.7% while trabecular vBMD decreased 1.7%.[50] None of the studied factors in this thesis displayed any associations with cortical vBMD, whereas both smoking (paper III) and prevalent fractures (paper IV) were mainly related to the trabecular vBMD. Smoking, however, only showed significant associations

with the trabecular vBMD of the tibia, the only appedicular site decreasing in vBMD. The decrease among subjects starting to smoke was more than three times that of the decrease among nonsmokers, and cross-sectionally at age 24, the trabecular vBMD of the tibia was 7.0% lower in smokers than in nonsmokers. This deficit, although beeing cross-sectional data, corresponds to several times the magnitude of the reported loss of trabecular vBMD at the tibia in the whole GOOD cohort between 19 and 24 years.

Ohlsson and Darelid et al further reported that the 5-year increase in aBMD of the radius in the GOOD cohort was also due to an increased size of the bone, where the cortical thickness (3.8%) and cross-sectional area (3.1%) displayed increments, which in turn was due to a decreasing endosteal circumference (-2.3%). At the tibia, essentially the corresponding changes were reported except for proportionally larger increases in size.[50] Of the studied factors in this thesis, hip fracture in a grandfather (paper I) and maternal age (paper II) were mainly associated with a lower cortical bone size. In these factors, both studied at age 19, the lower cortical cross-sectional area was likely the result of both lower periosteal and endosteal circumference. Whether these determinants are negatively associated with the continous acquisition of bone size, by opposing the normal age-dependent cortical consolidation, during the third decade of life remains to be studied. For maternal age though, the report of an increased fracture incidence with increased maternal age in a Brasilian cohort from birth to age 11,[100] indicates that the bone could have been smaller already from childhood. The majority of the measurements of the appendicular skeleton seems to still be under development in the GOOD-population, except for trabecular vBMD at the tibia which had started to decline.[50] A longitudinal study by Khosla et al,[52] including a subsample of 88 men between 22-39 years of age, demonstrated increases in aBMD at the mid-distal radius and ulna as measured by DXA at baseline, 2 years, and at 4 years. Other longitudinal studies using pQCT in young men are scarce, but in one study by Riggs et al,[126] the authors reported rather stable cortical and trabecular vBMD of both the radius and tibia between 20-29 years of age. Between age 30 and 39 the trabecular vBMD started to decline at both the radius and tibia, but not until the fifth decade of life the vBMD of the cortex displayed significant decrements in the radius and tibia. No geometrical parameters were presented in this study.

Bone acquisition on microstructural level

There are, so far, only one study with longitudinal data investigating the development of microstructural traits as derived by HR-pQCT during bone

mass accrual. In this study, by Nishiyama et al,[127] including 398 subjects (9-22 years) of which 186 were boys, two or three annual measurements of the distal radius and tibia were performed in a majority of the subjects. The investigation of changes across puberty was, however, analysed cross-sectionally, by comparing means in prepubertal subjects (Tanner 1) with subjects in early puberty (Tanner 2 and 3), peripuberty (Tanner 4) and postpuberty (Tanner 5). No significant increase was observed in trabecular BV/TV between prepubertal stage and any other of the pubertal stages at neither the radius nor tibia in males. This had previously been demonstrated at the tibia in a cross-sectional study including 146 men aged 15-20 years by Burrows et al,[128] where no significant differences were seen for any of the trabecular variables at the distal tibia. In contrast, Kirmani et al[129] demonstrated increases of trabecular BV/TV and thickness at the distal radius in a cross-sectional study, from late puberty and onward in 61 boys (6-21 years), which was also observed cross-sectionally by Wang et al,[130] in 69 boys (5-18 years). In paper III, men who were smokers at both baseline and 5-year follow-up, had substantially lower trabecular BV/TV of the tibia at age 24 years as a consequence of a lower trabecular thickness (8.9%), which was also the case at the radius (6.3%), than nonsmokers. Although the evidence of the normal development of trabecular microstructure is diverging, two of the aforementioned studies demonstrated late puberty as a period of increased accrual of trabecular thickness. Whether this increased accrual continues also between the age of 19 and 24 is unclear, but the results in paper III implies that smoking negatively affects trabecular thickness, possibly during, or slightly after, the period of its most intense development. None of the aforementioned studies presents fracture data, but Chevalley et al reported that boys with a mean age of 15.2 years with prevalent fractures had lower trabecular BV/TV and trabecular number than age-matched controls.[95] In paper IV we reported that trabecular BV/TV was more strongly associated with prevalent fractures than aBMD of the radius at age 24 years, and this was partly the result of a reduced trabecular thickness. This suggests that a prevalent fracture in childhood could be an indicator of an impaired development of the trabecular bone, persisting into the third decade in life.

As for the cortical parameters, Nishiyama et al also reported that cortical vBMD was significantly higher at both the radius and tibia at peri- and postpuberty, but not early puberty, as compared to prepuberty.[127] In Burrows's study, both cortical vBMD and cortical thickness of the tibia had their largest increases from age 17 and onward,[128] which was also the case at the radius in the study by Kirmani et al.[129] Cortical porosity of the radius was found to be transiently decreasing around the time of peak height velocity (PHV), which was accompanied by a sharp decrease in the fraction of load

carried by the cortical bone in the study by Kirmani. As suggested by the authors, this coincides with the age where the peak in distal forearm fractures occur, and they hypothesized that this could be part of the underlying cause. Interestingly Wang et al,[130] also observed a transient decrease in cortical thickness and lower cortical vBMD at the distal radius around PHV, and hypothesized that this was due to an increased cortical porosity. The authors hypothesized that this was due to the rapid increase in longitudinal growth during PHV which, because of the rapidity, precedes the normal corticalisation of trabecular bone, resulting in a transient increase of pores in the cortex. In paper IV, we found no associations between prevalent fractures and low vBMD of the cortex or high porosity at 24 years of age at the radius, whereas low cortical porosity of the tibia was actually associated to an increased fracture prevalence when adjusting for covariates. The interpretation of this finding must however be done with caution since the method used was associated with large CV values of 15.9% at the radius and 5.5% at the tibia.

CONCLUSION

Increased knowledge of the mechanisms behind the development of osteoporosis is of major significance because of high fracture incidence rates, human suffering in terms of increased mortality and morbidity, decreased mobility and autonomy, and financial burden for society. More profound understanding of the determinants of bone mass accrual and factors of importance for fractures during growth and during young adulthood could lead to enhanced methods of detecting subjects with low bone mass, which could enable measures to improve bone health and prevent fractures. In the present thesis two novel risk factors for low peak bone mass in men have been identified, including hip fracture in a male second-degree relative, which primarily affected the cortical bone size, and advancing maternal age, which could be an important factor for fracture incidence in populations with postponed childbearing. Altered smoking behaviour has for the first time been studied longitudinally in relation to bone mass development in young men, and the results from this thesis suggest that smoking has deleterious effects on bone mass development in young adulthood. Finally, the prevalence of a fracture has in a large population of young men been shown to be primarily associated to impaired trabecular microstructure and estimated variables of bone strength, and should thus be regarded as a risk factor of a suboptimal peak bone mass acquisition.

FUTURE PERSPECTIVES

The present thesis raises several new hypothesis to be dealt with, and the present material with detailed information about the bone mass in young men around the time of peak bone mass enables several future studies within and outside the used cohort. Future suggested studies include:

- To examine the future longitudinal development of bone mass in relation to maternal age and hip fracture in a grandparent.
- To investigate both prevalence and incidence of fractures in young men in relation to hip fracture in a grandparent and maternal age by linking the study databases to the patient registers.
- To investigate the importance of PBM on future fracture incidence, assessed using the patient registers, in an aging GOOD cohort.
- To longitudinally investigate the continuous effect of smoking on microstructural bone development by performing a 10-year follow-up in the GOOD cohort.

There are also possibilities to perform large register studies on the swedish population. Some of the ideas are to:

- Link the entire Swedish medical birth register to the patient registers, and thereby investigate whether an increasing maternal age is related to an increased fracture prevalence in the Swedish population.
- Use the multigeneration register to link, for example, the total Swedish population in a certain age group to their grandparents, and use the patient registers to investigate whether there is a difference in fracture rates between the grandchildren of grandparents with and without prevalent fractures.

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