DETERMINANTS OF BONE HEALTH IN MIDDLE AGED AND OLDER MEN: THE IMPACT OF HORMONES, LIFESTYLE AND CHILDHOOD FRACTURE

A thesis submitted to the University of Manchester for the degree of Doctor of Philosophy (PhD) in the Faculty of Medical and Human Sciences

2014

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Contents list

List of abbreviations 4
Abstract 6
Declaration 7
Copyright statement 10
Acknowledgments 11
Statement 12
Chapter 1 - Introduction 15
  1.1 Osteoporosis 15
Chapter 2 - Study cohorts and analysis 20
  2.1 The European Male Ageing Study 20
    2.1.1 Recruitment 20
    2.1.2 Postal questionnaire 23
    2.1.3 Interviewer-assisted questionnaire 24
    2.1.4 Physical performance and anthropometry 24
    2.1.5 Hormone measurements 24
    2.1.6 25-hydroxy- and 1,25-dihydroxyvitamin D 25
    2.1.7 Bone turnover measurements 25
    2.1.8 Quantitative heel ultrasound 26
    2.1.9 Dual-energy x-ray absorptiometry 26
    2.1.10 Peripheral quantitative computed tomography 26
    2.1.11 Statistical analysis: EMAS publications 1-5 27
  2.2 The European Prospective Osteoporosis Study 28
    2.2.1 Recruitment 28
    2.2.2 Bone mineral density 29
    2.2.3 Statistical analysis: EPOS publication 6 30
Chapter 3 - Summary of published work 31
  3.1 Role of sex steroids on bone health in men: publications 1-3 31
    3.1.1 Background and aims 31
    3.1.2 Contribution to literature 34
      3.1.2.1 Bone turnover and bone density 34
      3.1.2.2 The role of oestradiol 34
      3.1.2.3 The role of testosterone 36
      3.1.2.4 The role of SHBG 37
3.2 Influence of vitamin D on bone health in men: publication 4
3.2.1 Background and aims 38
3.2.2 Contribution to literature 39
3.2.2.1 Association with age, season and centre 40
3.2.2.2 Interrelationship 40
3.2.2.3 Influence of 1,25(OH)2D 41
3.2.2.4 Influence of 25(OH)D 42
3.2.2.5 Influence of PTH 42
3.3 Influence of lifestyle factors on bone health in men: publication 5
3.3.1 Background and aims 44
3.3.2 Contribution to literature 44
3.3.2.1 Influence of age 45
3.3.2.2 Influence of physical activity 46
3.3.2.3 Influence of smoking and alcohol 46
3.4 Influence of childhood fracture on BMD and future fracture: publication 6
3.4.1 Background and aims 48
3.4.2 Contribution to literature 49
3.5 Implications for future work 52

Chapter 4 - Scientific impact 54
References 56
The Publications 74
Publication 1 74
Publication 2 75
Publication 3 76
Publication 4 77
Publication 5 78
Publication 6 79
## List of abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,25(OH)_2D</td>
<td>1,25-dihydroxyvitamin D</td>
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<tr>
<td>25(OH)D</td>
<td>25-hydroxyvitamin D</td>
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<tr>
<td>AR</td>
<td>androgen receptor</td>
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<tr>
<td>B_cTX</td>
<td>beta C-terminal telopeptide cross-linked telopeptide</td>
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<tr>
<td>BMD</td>
<td>bone mineral density</td>
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<tr>
<td>BMD_a</td>
<td>areal bone mineral density</td>
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<tr>
<td>BMC</td>
<td>bone mineral content</td>
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<tr>
<td>BMI</td>
<td>body mass index</td>
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<tr>
<td>BUA</td>
<td>broadband ultrasound attenuation</td>
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<tr>
<td>CI</td>
<td>confidence interval</td>
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<tr>
<td>CSMA</td>
<td>cross-sectional muscle area</td>
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<tr>
<td>CV</td>
<td>coefficient of variation</td>
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<tr>
<td>DHT</td>
<td>dihydrotestosterone</td>
</tr>
<tr>
<td>DXA</td>
<td>dual energy x-ray absorptiometry</td>
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<tr>
<td>E_2</td>
<td>oestradiol</td>
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<tr>
<td>ER</td>
<td>oestrogen receptor</td>
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<tr>
<td>EMAS</td>
<td>European Male Ageing Study</td>
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<td>EPOS</td>
<td>European Prospective Osteoporosis Study</td>
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<tr>
<td>FSH</td>
<td>follicle stimulating hormone</td>
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<tr>
<td>HR</td>
<td>hazard ratio</td>
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<tr>
<td>IGF</td>
<td>insulin growth factor</td>
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<tr>
<td>LOWESS</td>
<td>locally-weighted scatter plot smooth</td>
</tr>
<tr>
<td>LH</td>
<td>luteinizing hormone</td>
</tr>
<tr>
<td>MS</td>
<td>mass spectrometry</td>
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<tr>
<td>OR</td>
<td>odds ratio</td>
</tr>
<tr>
<td>P1NP</td>
<td>N-terminal propeptide of type 1 procollagen</td>
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<tr>
<td>PASE</td>
<td>physical activity score for the elderly</td>
</tr>
<tr>
<td>PPT</td>
<td>physical performance test</td>
</tr>
<tr>
<td>PQ</td>
<td>postal questionnaire</td>
</tr>
<tr>
<td>pQCT</td>
<td>peripheral quantitative computed tomography</td>
</tr>
<tr>
<td>PTH</td>
<td>parathyroid hormone</td>
</tr>
<tr>
<td>QoL</td>
<td>quality of life</td>
</tr>
<tr>
<td>QUI</td>
<td>quantitative ultrasound index (QUI)</td>
</tr>
<tr>
<td>QUS</td>
<td>quantitative ultrasound</td>
</tr>
<tr>
<td>RIA</td>
<td>radioimmunoassay</td>
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<tr>
<td>SD</td>
<td>standard deviation</td>
</tr>
<tr>
<td>SF36</td>
<td>short form (36) health survey</td>
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<tr>
<td>SHBG</td>
<td>sex hormone binding globulin</td>
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<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<td>--------------</td>
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<tr>
<td>SOS</td>
<td>speed of sound</td>
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<tr>
<td>SSI</td>
<td>stress strain index</td>
</tr>
<tr>
<td>T</td>
<td>testosterone</td>
</tr>
<tr>
<td>UK</td>
<td>United Kingdom</td>
</tr>
<tr>
<td>USA</td>
<td>United States of America</td>
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Abstract

Background & Aim: Osteoporosis is an important clinical and public health problem through its association with age-related fractures. Compared to women, much less is known about what factors determine age-related bone loss in men. The aim of the work presented in this thesis was to examine the impact of the main steroid hormones, (sex hormones & vitamin D), lifestyle factors and prior fracture on bone health in middle age and elderly men in Europe.

Methods: Data presented in this thesis was derived from two large multicentre observational studies: the European Male Ageing Study (EMAS) and the European Prospective Osteoporosis Study (EPOS). In EMAS 3,369 men aged between 40 and 79 years were recruited from 8 European centres for participation in a study of male ageing. They completed a postal questionnaire which included questions concerning lifestyle and were invited to attend for quantitative ultrasound (QUS) of the heel, from which the parameters broadband ultrasound attenuation (BUA) and speed of sound (SOS) were obtained, a questionnaire including measures of physical activity, assessment of physical performance and a fasting blood sample from which the bone markers serum N-terminal propeptide of type 1 procollagen (P1NP) and crosslinks (β-cTX), total testosterone (T), total oestradiol (E₂) and sex hormone-binding globulin (SHBG), 25-hydroxyvitamin D (25(OH)D) and 1,25-dihydroxyvitamin D (1,25(OH)₂D) were measured. Dual energy x-ray absorptiometry (DXA) of the hip and lumbar spine and peripheral quantitative computed tomography (pQCT) of the radius at the distal (4%) and midshaft (50%) sites was performed in a subset of two centres. In EPOS, 6,656 men and 7,203 women aged 50 years and over were recruited from population registers in 32 centres. Subjects completed an interviewer administered questionnaire that included questions about previous fractures. Subjects were followed prospectively for a median of 4 years to determine the new occurrence of fractures. A subsample of subjects had bone mineral density measurements performed.

Key Results: Based on data from EMAS, free T and both free and total E₂ were positively related to the QUS parameters BUA and SOS, while SHBG concentrations were negatively associated. Total and free E₂ were negatively associated with β-cTX though not P1NP while PTH was positively associated with both β-cTX and P1NP. Higher levels of both bone markers were significantly associated with lower QUS parameters and lower DXA-assessed bone density at the total hip and lumbar spine. 25(OH)D was negatively while 1,25(OH)₂D positively associated with bone parameters. Higher levels of physical activity and lower physical performance were associated with both higher BUA and SOS. Smoking was associated with lower QUS parameters, while there was a U shaped association with frequency of alcohol consumption. A recalled history of any childhood fracture or forearm fracture was not associated with either bone mass in later life or an increased risk of fracture in men as well as women.

Conclusion: Steroid hormones, particularly oestrogen & vitamin D are associated with bone health in middle age and older men. Modification of lifestyle, including increasing physical activity and stopping smoking may help optimise bone strength and reduce the risk of fracture in men. In assessment of future fracture risk a history of childhood fracture does not appear to be important.

The University of Manchester
Stephen Richard Pye
Doctor of Philosophy
Determinants of Bone Health in Middle Aged and Older Men: The Impact of Hormones, Lifestyle and Childhood Fracture 2014
Declaration

The University of Manchester
PhD by published work: Candidate Declaration

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FACULTY OF MEDICAL AND HUMAN SCIENCES

DETERMINANTS OF BONE HEALTH IN MIDDLE AGED AND OLDER MEN: THE IMPACT OF HORMONES, LIFESTYLE AND CHILDHOOD FRACTURE

1. I am the lead or joint lead author of publications 4-6 and the second author on publications 1-3, making substantial contributions to drafting the manuscripts for all these publications. I designed and performed the statistical analysis for all publications.

Publication 1:
As a member of the analysis and publication writing team, I contributed to the scientific ideas that lead to the hypotheses tested in this publication. I designed, performed, presented and described all the statistical analysis / results and worked closely with the lead author, prepared the first draft and made substantial contributions to the writing thereafter.

Publication 2:
I was involved in the scientific discussions that resulted in the ideas examined in this publication. I planned, performed, presented and described all the statistical analysis / results and worked closely with the lead author, making substantial contributions to the writing. I prepared the final manuscript.
Publication 3:

As a member of the study team I was heavily involved in the discussions that lead to the research question. I planned, performed, presented and described all the statistical analysis / results and worked closely with the lead author, making substantial contributions to the writing. I prepared the final version of the manuscript, incorporating the comments from all the coauthors and was responsible for submitting this to the journal.

Publication 4:

I was involved in scientific discussions that lead to this publication. I designed, conducted, presented and described all the statistical analysis / results and was the joint lead author. I prepared the first draft and made substantial contributions to the drafting of the manuscript including preparing the final version.

Publication 5:

As part of the analysis team, I took part in the discussions leading to the ideas presented in this publication. I planned, performed, presented and described all the statistical analysis / results and was the lead author. I prepared the manuscript.
Publication 6: 

I was involved in the discussions leading to the scientific questions examined in this publication. I designed, performed, presented and described all the statistical analysis / results and as the lead author, prepared and submitted the manuscript.

2. All of the work presented here was completed whilst I have been a member of staff at The University of Manchester.

3. None of the work presented here has been submitted in support of a successful or pending application for any other degree or qualification.

I confirm that this is a true statement and that subject to any comments above, the submission is my own original work.

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Date:....................................................
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Acknowledgements

I would like to thank all those who contributed to the publications presented here. Thanks also go to the subjects and researchers of the EMAS and EPOS projects without whom this work would not have been possible. My sincere thanks go to my supervisor, Professor Terry O’Neill, for his guidance, encouragement and patience. Finally I would like to thank my girlfriend Susannah James for her incredible support without which I would not have been able to complete this thesis.
Statement

i. Particulars of the candidate’s degrees, other qualifications and research experience:

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- 11 oral and 23 poster presentations at national and international conferences
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- Module in applied statistics Oct-Dec 2002
- Module in advanced statistics Jan - March 2003
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Awards:
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ii. A complete and numbered list of the publications submitted (grouped according to subject and type):

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**Publication 2:**

**Publication 3:**

**Publication 4:**
*authors contributed equally

**Publication 5:**

**Publication 6:**
**Pye SR**, Tobias J, Silman AJ, Reeve J, O'Neill TW on behalf of the EPOS Study Group. Childhood fractures do not predict incident fractures: results from the
iii. An overall summary of the aims and achievements of the work.

Chapter 1. Introduction

This chapter provides a short background to the topic area of osteoporosis, setting into a broader context the work presented in the thesis.

1.1 Osteoporosis

Osteoporosis is a major public health problem through its main clinical consequence: fracture. Such fractures are serious and lead to severe mortality and morbidity, a significant burden on society in general, and substantial health care costs. Over 300,000 fractures related to osteoporosis occur each year in the UK at an estimated cost of £1.73 billion (1-4). The most frequent fractures due to osteoporosis are fractures in areas of the skeleton containing substantial amounts of trabecular bone such as the hip, spine and wrist though most fractures which occur over the age of 50 years are at least in part related to osteoporosis (5).

Fracture incidence has two peaks, one in the young and one in the elderly. In young people, fractures usually occur in the long bones and are associated with substantial trauma. The incidence of fracture is higher in boys than girls, and peaks slightly later in boys (13-15 years of age) compared to girls (10-12 years of age) (6). Fractures in children are common and at their peak (boys 3%, girls 1.5%) are surpassed only among women aged 85 and never in men (6).
The incidence of osteoporotic fractures increases with age from around 50 years though the pattern of increase in incidence with age varies by fracture site (7). Fractures occur as a result of an interaction between reduced bone strength, the main determinant of which is bone mass, and trauma, most frequently a fall from a standing height or less. The increased risk of fractures with age can be explained in part on the basis of an age related decline in bone mass and strength and also an increase in risk of falls with age (5).

In most populations, hip fracture incidence increases exponentially with age. Most hip fractures occur following a fall from standing height. It has been estimated that in 1990 there were 1.66 million hip fractures worldwide, representing 1,197,000 fractures in females and 463,000 among males, and that this figure will rise to 6.3 million by 2050 due to an increasing number of elderly people in the population (8). In Western populations, among individuals above 50 years of age, there is a female preponderance of hip fracture, with a female-to-male incidence ratio of approximately 2:1. Overall, around 98% of hip fractures occur in people greater than 35 years of age, and 80% occur in women (9). Incidence rates vary considerably according to geographic area and race. In Europe, hip fracture rates vary seven-fold between countries, with the highest rates observed in Scandinavians (10). A study from 1989 found the age-adjusted 1-year cumulative incidence of hip fracture in Norway was 903/100,000 for women and 384/100,000 for men (11).

It has been difficult to obtain accurate estimates of vertebral fracture, partly because it is often asymptomatic and also due to the lack of consensus regarding how to define a vertebral fracture (12). However, the advent of description of morphometric and semi-quantitative visual techniques has
enabled a number of studies to report on the prevalence of vertebral fracture. Only about a third of all vertebral deformities noted on radiographs come to medical attention, and less than 10% necessitate admission to hospital (13). In the European Vertebral Osteoporosis Study, one in eight women and men aged 50 years and over had evidence of vertebral deformity (14). Prevalence of vertebral deformity increased steadily with age in both sexes, although the gradient was steeper for women. There was a three-fold variation in the occurrence of deformity across Europe, and up to a two-fold variation in centres within individual countries, perhaps reflecting a combination of environmental and genetic factors. The risk of vertebral deformity among men was significantly elevated in those with high levels of physical activity, suggesting the aetiological importance of trauma. By contrast, women with higher levels of customary physical activity have a reduced risk of deformity (15).

Distal forearm (wrist) fracture almost always occurs as a consequence of a fall onto an outstretched hand. In Caucasian women, the incidence increases linearly between the ages of 40 and 65 and then plateaus, while in men the incidence remains constant between the ages of 20 and 80 years (7). There is a pronounced gender difference, estimated to be 4:1 in favour of women. Recent data from Dorset, UK, showed that among women the incidence of distal radius fracture rose from a pre-menopausal baseline of 10 per 10,000 of the population per year to a peak of 120 per 10,000 of the population per year over 85 years (16). The plateau with age in women may be due to mode of falls; later in life a woman is more likely to fall onto a hip than an outstretched hand as her neuromuscular coordination deteriorates. The incidence rates of
proximal humeral, pelvic, and proximal tibial fractures also rise steeply with age, and are greater in women than men (7).

Bone mass can be assessed non-invasively using bone densitometry and data from many observational studies show an inverse relationship between the level of bone mass (typically measured by dual energy X-ray absorptiometry (DXA) and recorded as an areal density, bone mineral density (BMDa)) and fracture. The risk of osteoporotic fracture increases continuously as BMDa declines in women with a 1.5- to 3-fold increase in risk of fracture for each standard deviation (SD) fall in BMDa (17). Other factors which impact on bone strength and which are independent of bone mass include bone size and shape (geometry), the microarchitecture of the bone, and also the activity of the cells responsible for the renewal of bone (bone turnover). Methods have been established to quantify these factors including quantitative ultrasound (QUS) and quantitative computed tomography (QCT) to assess structural factors and urine or serum markers of bone turnover to assess underlying cellular activity, though relatively less is known about these factors and their determinants compared to bone mass (5).

Following its first recognition as a clinical syndrome in the late 1940’s, osteoporosis was considered to be a condition predominantly affecting post-menopausal women (18). In part this was because of the important role identified for menopausal oestrogen decline in the pathogenesis of osteoporosis in women but also, because fractures increase with age and because women live longer than men, the observation that the majority of fractures occurred in women. As a consequence much more attention and research efforts have focused on osteoporosis in women. It has become apparent though in the last
decade in particular that osteoporosis is a significant clinical and public health problem in men also. The lifetime risk of sustaining a fracture from the age of 50 years in men in the UK has been estimated at 20% and at any age the incidence of fracture is just half that as in women (5). Furthermore outcome following fracture tends to be worse in men than women with greater morbidity and, at least in the case of hip fracture, greater mortality (19).

The last decade as a consequence has seen a substantially greater research focus on bone health in men with now improved knowledge and understanding including the assessment of bone health in men and also the underlying causes and pathogenesis of bone fragility (19). Many of the factors linked with bone fragility in women have been found to be linked with bone fragility in men; thus for example the risk of fracture related to bone loss assessed using DXA has been shown to be similar in men and women (5). Much though remains to be learned including the impact particularly of steroid hormones and particularly sex hormones on bone health, including not just mass, but other structural and bone turnover parameters. Also the impact of lifestyle factors on the non-mass related structural components of bone in men remains uncertain. Such data are important; with the increasing size of our ageing population and the improving longevity of men, osteoporosis is set to increase substantially in men over the next 50 years (5). A better understanding of the determinants of bone health is important in both in helping better identify those who are at risk of fracture and also in informing the development of targeted and population wide strategies for prevention.
Chapter 2. Study cohorts and analysis

This chapter describes the study cohorts that were used to address the research questions posed and which are presented in this thesis. It includes also a summary of the statistical methodologies used in the analyses. Publications 1 to 5 included in the thesis used data from the European Male Ageing Study (EMAS) while publication 6 used data from the European Prospective Study of Osteoporosis (EPOS).

2.1 The European Male Ageing Study (EMAS)

2.1.1 Recruitment

EMAS is a multi-centre, population based, prospective survey funded by the European Commission fifth Framework Programme, ‘Quality of Life and Management of Living Resources’. There were eight participating centres: Florence (Italy), Leuven (Belgium), Łódź (Poland), Malmö (Sweden), Manchester (UK), Santiago de Compostela (Spain), Szeged (Hungary) and Tartu (Estonia). Participating centres were recruited on the basis of their interest and expertise in male health, access to a population based sample and facilities to perform an epidemiological survey. The cross-sectional survey of a random population sample of men aged 40-79 years was completed in 2003-2005; the prospective phase in 2010. The publications presented in this thesis are based on analysis of data from 3,369 men who were recruited to the baseline survey. Ethical approval for the study was obtained in each of the centres in accordance with local practice and requirements.
Each centre was invited to obtain details of a local population based register, which included information about name, gender, date of birth and address. The choice of sampling frame was based on availability and representativeness of the local adult population within each centre. Stratified random sampling was used with the aim of recruiting equal numbers of men into each of four 10-year age bands (40–49, 50–59, 60–69 and 70–79 years). There were no specific exclusion criteria apart from subjects being able to provide written, informed consent.

Subjects were invited by letter of invitation to attend for a screening visit at a local clinic. The letter included an information sheet about the study and a short postal questionnaire (PQ) about lifestyle factors, which subjects were asked to complete even if they declined further participation. The purpose of this was to obtain information about men who subsequently declined to participate in the main study. To help recruitment, centres were encouraged to advertise the study in the local news media. Subjects were re-contacted usually within 4 weeks, if they did not reply following the first letter. This approach was followed in all but one centre (Szeged) where second contacts were not undertaken. Additional contacts (beyond 4 weeks) were not attempted as it was considered less likely that such subjects would be retained in a longitudinal study. For those subjects who had not replied after two contacts, a sample of subjects were contacted by telephone inviting them to verbally answer a series of questions taken from the PQ, i.e. self-rated health, age at leaving full-time education, time typically spent walking or cycling each day and smoking status (never, past or current). For some subjects, such contact prompted entry into the study and they were then included as participants (20).
The main survey, which was conducted at a local clinical facility, involved an interviewer assisted questionnaire (IAQ), assessment of height and weight, a fasting blood sample (before 10.00am) and a number of physical performance measures.

In selecting the questionnaire instruments, preference was given to those that had been previously translated and validated in each of the participating centres’ languages. If this was not possible, and to reduce errors due to language differences, questionnaires were initially translated from the original English version to the local language by a professional translator. The translated questionnaires were then sent to each centre where they were back-translated into English and checked for authenticity. Further modification of the translated questionnaires by each centre was then carried out if required. During the piloting phase, any inconsistencies were reported to the coordinating centre and modifications made to the questionnaire (20).

The overall response rate for full participation in EMAS was 40%, with the mean centre response rate slightly higher at 43%. Response rate varied by centre from 24 to 60% and there was some evidence that those who had been recruited from a health register (Florence, Tartu and Manchester) had a slightly higher response rate than those recruited from an electoral or other register.

Adjusting for those who had died or moved (in centres in which it was possible to identify them) the corrected overall response rate was slightly higher at 41%, and the mean centre response rate at 45%. Restricting the analysis to centres who undertook follow-up contact of initial non-responders, the overall response rate increased from 40 to 44% and the mean centre response rate from 43 to
46%. Response rates were highest among those aged 50-59 years (45%) and declined with age with the lowest in the oldest age decade (34%) (20).

Comparing the characteristics of the participants versus the non-participants, men who completed and returned the PQ but did not subsequently participate in the main study (n = 594) were older (62.8 vs. 60.0 years; p < 0.001), spent less time in full-time education (19.1 vs. 20.9 years; p < 0.001), were more likely to rate their general health as fair or poor (38 vs. 33%; p = 0.038), were more likely to spend ‡½ h walking or cycling per day (70 vs. 65%; p = 0.021) and reported experiencing less pain lasting at least 1 day in the past month (44 vs. 59%; p < 0.001) than those who participated in the full study. There was, however, no difference between these groups in the number of morbidities, current and past smoking, and frequency of alcohol consumption. Compared to full study participants, men who took part in the telephone survey of non-responders (n = 361) left full-time education earlier (18.7 vs. 20.9 years; p < 0.001) and were more likely to be current smokers (33 vs. 21%; p < 0.001). No differences were found between the full participants and those who took part in the telephone survey regarding general health, time spent walking or cycling per day, or the proportion who had ever smoked (20).

2.1.2 Postal questionnaire (PQ)

The PQ included questions concerning time spent walking or on a bicycle out of doors each day (response set = none / less than 30 minutes / 30 minutes to 1 hour / more than 1 hour), smoking - both ever and currently, alcohol consumption in the previous year (response set = every day / 5-6 days per week / 3-4 days per week / 1-2 days per week / less than once a week / not at all).
There was a question also about prior fracture since the age of 25 years (response set = no / yes / don’t know). Subjects were asked also whether they were currently being treated for any of a list of fourteen morbidities: heart conditions, high blood pressure, pituitary disease, testicular disease, chronic bronchitis, asthma, peptic ulcer, epilepsy, diabetes, liver conditions, kidney conditions, prostate disease, adrenal disease, thyroid disease.

2.1.3 Interviewer-assisted questionnaire (IAQ)
This questionnaire included the physical activity scale for the elderly (PASE) and the SF36 quality of life questionnaire (21, 22).

2.1.4 Physical performance and anthropometry
A number of performance measures were undertaken including the Tinetti assessment of balance and gait which included the time taken to go from a sitting to a standing position (five times), and also Reuben’s physical performance tests (PPT) which included the time taken to walk 50 feet (23, 24). Height and weight were measured in a standardised fashion.

2.1.5 Hormone measurements
Measurement of T and E₂ were carried out by gas chromatography mass spectrometry (GC-MS) at a laboratory in Quebec, Canada as described in Labrie et al. (25, 26). SHBG, which plays a key role in the transport of sex hormones in plasma and its concentration is a major factor regulating their distribution between the protein-bound and free states, was measured in Florence, Italy by the Modular E170 platform electrochemiluminescence immunoassay (Roche
Diagnostics, Mannheim, Germany). The free and bio-available (non-SHBG-bound) T and E₂ levels were derived from total hormone, SHBG and albumin concentrations using mass action equations and associations constants of Vermeulen et al. and Van Pottelbergh et al. (27, 28). In addition, samples were transported in frozen state to a single laboratory at the University of Santiago de Compostela, Spain for measurement of insulin growth factor 1 (IGF-1) by chemiluminiscence. Further details can be found in publication 1.

2.1.6 25-hydroxy- and 1,25-dihydroxyvitamin D measurements
Serum 25(OH)D levels were determined using radioimmunoassay (RIA kit: DiaSorin, Stillwater, MN, USA). 1,25-(OH)₂D₃ was measured by liquid chromatography tandem mass spectrometry (LC-MS/MS) as a lithium adduct according to the method described by Casetta et al. (29). Both vitamin D assays were conducted in a laboratory at the University of Leuven, Belgium. Further details can be found in publication 4.

2.1.7 Bone turnover measurements
To assess bone resorption, serum beta C-terminal telopeptide cross-linked telopeptide (β-cTX) was measured on the Elecsys 2010 automated analyzer (Roche Diagnostics GmbH, Mannheim, Germany) using the β-Crosslaps/serum reagents (30). To evaluate bone formation, measurements were performed on the Elecsys 2010 with a 2-site assay using monoclonal antibodies raised against intact human P1NP purified from human amniotic fluid. Both bone turnover measurements were conducted in a laboratory at the University of Leuven, Belgium. Further details can be found in publication 3.
2.1.8 Quantitative heel ultrasound (QUS)
Quantitative ultrasound of the heel was performed using the Sahara Clinical Sonometer (Hologic, Bedford, Massachusetts) using a standardised protocol. Each centre used the same machine model, and each calibrated daily with the physical phantom provided by the manufacturer. Outputs included broadband ultrasound attenuation (BUA) and speed of sound (SOS). Further details can be found in publication 5.

2.1.9 Dual-energy x-ray absorptiometry (DXA)
Areal bone mineral density (BMDₐ) scans were carried out on men in two centres, Manchester and Leuven, using dual-energy x-ray absorptiometry (DXA) QDR 4500A devices (Hologic, Inc, Waltham, MA, USA). BMDₐ was measured at the lumbar spine (L1 to L4) and proximal femur (total region). Both devices were cross-calibrated with the European Spine Phantom (31).

2.1.10 Peripheral quantitative computed tomography (pQCT)
Peripheral QCT measurements of the non-dominant radius were made in men in two of the centres, Manchester and Leuven, using XCT-2000 scanners (Stratec, Pforzheim, Germany). At the distal (4%) site total and trabecular BMD (mg/cm³) and bone cross sectional area (mm²) were measured (voxel size 0.4mm); the slice location at the 4% and 50% site was more distal in Leuven compared to Manchester; the reference line was placed at the distal border of the radial endplate in Leuven, in Manchester the line was placed to bisect the lateral border of the endplate these differences result in a scan site difference
approximately 1-2mm between centres. At the diaphysis (50% site, voxel size 0.6mm), cortical BMD (mg/cm$^3$), bone mineral content (BMC mg/mm), total, cortical and medullary areas (mm$^2$), cortical thickness (mm), stress strain index (SSI, mm$^3$) and muscle cross-sectional area, as a proxy for muscle strength (CSMA, mm$^2$), were measured. SSI provides a measure of a bone’s torsional strength (32). Further details are included in publication 2.

2.1.11 Statistical analysis: EMAS publications 1-5

In relation to data from EMAS, descriptive statistics were used to summarize subject characteristics. The associations between exposure and outcome variables were assessed visually using scatter plots, super-imposing linear lines and lowess (LOcally-WEighted Scatter plot Smooth) curves to gain an idea about the nature of any underlying association. Least squares regression modeling was used to examine the influence of the individual exposures of interest on bone health with the various bone structural and turnover parameters as the dependent variables as appropriate. The type of model depended on the outcome variable; linear regression for continuous data and logistic regression for categorical data. Continuous exposure variables were analysed either as untransformed and / or after categorization (usually into tertiles or quintiles) to examine for nonlinear or threshold effects. For some analyses, variables were transformed into z scores facilitating comparisons of effect size. Potential confounders were tested for association with the outcome and the risk factor of interest and were included in the main models if found to be significantly associated with both. Adjustment for confounding was usually performed in a sequential manner, starting with age and centre, then adding height / weight /
BMI, and then adding any additional confounders. To allow more direct comparison with some key previous studies, the cohort was stratified by age for some analyses: less than and greater than 60 years. For all linear regression models, the distribution of the residuals was assessed by plotting quantiles of the standardised residuals against quantiles of a normal distribution, visually assessing if the plot deviated from a straight line and then statistically testing for deviation from normality using the Shapiro-Wilk test. The results were expressed depending on the statistical model as either $\beta$ coefficients or odds ratios (OR) and 95% confidence intervals (CI). All statistical analyses were performed using the statistical package Stata, version 11.2 (www.stata.com).

2.2 The European Prospective Osteoporosis Study (EPOS)

2.2.1 Recruitment

EPOS was a multi-centre, population based prospective study, funded by the European Union to look at the distribution and determinants of osteoporotic fractures in different regions and populations of Europe. Almost 17,000 men and women of 50 years of age and over of were recruited from population registers in 36 European centres. Stratified sampling was used with the aim of recruiting equal numbers of men and women in each of six 5-yr age bands: 50-54, 55-59, 60-64, 65-69, 70-74, and 75 years and above. Subjects were interviewed using a structured interview that included questions about previous fractures. Subjects were asked “Have you ever suffered from a broken bone (fractures)?.” If yes, subjects were asked about the site of their previous fracture(s) (vertebral, hip, rib, forearm, other), number of fractures, the age of their first fracture (at each site), and the level of trauma (spontaneous,
minor, or major trauma) for that fracture. Lateral spinal radiographs were performed to ascertain prevalent vertebral deformities. The radiographs were evaluated morphometrically by one of three observers and the presence of vertebral deformity determined using the McCloskey-Kanis method (33). The subjects recruited in 29 centres were followed prospectively by annual postal questionnaire and in a further 3 centres by telephone or personal interview. Because of a low follow-up rate, data from one centre were subsequently excluded from the analysis. Subjects were asked to record details of any fractures sustained in the intervening period, including marking on a body manikin (included in a previously validated postal questionnaire) the position or site of their fractures (34). Fractures reported were verified at each of the participating centres by the principal investigator by review of radiographs, medical record, or subject interview. From these sources, contemporary data to confirm or refute the occurrence and site of fracture were not available in 9% of cases. In these cases, the site of fracture was determined from the area marked by the subject on the manikin (34).

2.2.2 Bone mineral density

21 centres were able to measure BMD at the hip and or the spine at baseline or during follow-up in subsamples of between 20% and 100% of their available participants using DXA. The densitometers in each centres were, with one exception (a Sopha fan-beam machine), pencil beam DXA machines made by Lunar, Hologic, or Norland. They were cross-calibrated using the European Spine Phantom (ESP) (35). 4807 (36%) from 19 centres had hip BMD (femoral
neck and/or trochanter) measurements, and 3998 (30%) from 14 centres had spine BMD measurements. Further details are included in publication 6.

2.2.3 Statistical analysis - EPOS publication 6

Descriptive statistics were used to summarize subject characteristics. Cox proportional hazards model was used to assess the predictive risk of childhood fracture on the risk of non-vertebral fractures sustained during the follow up phase of the study. Subjects contributed follow-up time (person years) from the date of the baseline survey until limb fracture, death, or the end of the study. In subjects who sustained more than one incident fracture of the same type during follow-up, the time to the first fracture event was used in the analysis. The results of this analysis were expressed as hazard ratios (HRs) and 95% CIs. Logistic regression was used to examine the association between self-reported childhood fractures and prevalent vertebral deformity (identified from morphometry at the baseline survey), with the results expressed as ORs and 95% CIs. All analyses were undertaken separately in men and women with adjustments made for centre. All statistical analyses were performed using the statistical package Stata, version 11.2 (www.stata.com).
Chapter 3. Summary of published work

This chapter outlines the background knowledge, aims, key results and also contribution to the literature of each of the publications presented in the thesis. The first section focuses on publications related to the role of sex steroids on bone health (publications 1-3), the second section the role of vitamin D (publication 4), the third section on the role of lifestyle factors in explaining bone health (publication 5) and the fourth, the role of prior childhood fracture as a predictor of future fracture (publication 6).

3.1 Role of sex steroids on bone health in men: publications 1-3

This section summarises the background, aims, key results and contribution to the literature of publications 1-3. These publications focus on the role of sex steroid hormones in influencing bone health and therefore are considered together.

3.1.1 Background and aims

Testosterone (T) is the principal circulating androgen in the body. It can be converted to oestradiol (E₂; the main oestrogen) by the enzyme aromatase or alternatively converted to dihydrotestosterone (DHT) by the enzyme 5α-reductase. Concentrations of sex hormones in serum are determined by synthesis in the gonads and adrenal glands which is controlled by hypothalamic-pituitary feedback via the gonadotropins follicle stimulating hormone (FSH) and luteinizing hormone (LH). The activity of sex hormones is restricted by their high affinity binding to sex hormone binding globulin (SHBG). In men, about 45%
of circulating T is bound to SHBG and only the non-bound (free) fraction is considered bioactive (36). The majority of this fraction is non-specifically bound to albumin and only around 2% of T and E₂ circulates freely and is considered to be bioavailable (36, 37).

Bone is an endocrine tissue that expresses both androgen and oestrogen receptors and also enzymes that metabolise steroids (38). Hence T can potentially affect bone either directly by stimulating the androgen receptor (AR) or indirectly by stimulating the oestrogen receptor (ER) after aromatisation to E₂ (39). Gonadotropins do not appear to have a direct effect on bone tissue (36).

There is good evidence from epidemiological studies that sex hormones, in particular E₂, influence bone health in men as well as women (38, 40-44). However, the effect of T on bone health is less well understood as results from previous studies conflict. For example, in a study of community-dwelling Australian older men aged 60 years and older, serum T was independently associated with the risk of osteoporotic fracture (45), while in a European study serum testosterone had no effect on fracture risk (46).

BMD₃, measured by dual energy X-ray absorptiometry (DXA), has been the most commonly used measure of bone health. There are limitations, however, in assessment of bone health using DXA - it tends to overestimate BMD in larger, and underestimate in smaller, bones. Furthermore bone strength and susceptibility to fracture is not only influenced by the bone mineral content but also bone shape and mineral distribution and the loading conditions to which the bone is subjected. Newer techniques such as peripheral quantitative computed tomography (pQCT) allow assessment of both bone geometry and
material properties including volumetric density. In contrast to age-related changes in DXA BMD in men there are relatively few data concerning change in BMD and bone geometry as assessed by pQCT with age. Furthermore, the impact that sex steroids may have on pQCT parameters - particularly bone geometry is not well understood. Khosla et al. (47, 48) has showed that E₂ was most consistently associated with BMD and geometry, measured by QCT, with the effect being more marked in elderly men as the age related decline in sex steroids becomes more relevant. Similarly in the MIcros cohort, E₂ was related to DXA BMDₐ cortical thickness and area (49).

Sex hormones could potentially influence bone health by affecting bone remodeling (50-52). Several cross-sectional studies in men have examined the associations between serum bone turnover markers and age, sex hormones and DXA-based BMD (53-58). In cohorts of older men, previous studies have shown higher levels of bone turnover markers to be associated with lower BMD (cross-sectionally), higher rates of bone loss and poorer bone microarchitecture, though there is few data linking bone turnover to risk of fracture (55, 57, 59-64). Data on the relationship between bone turnover and quantitative ultrasound (QUS) measurements of the heel are much more limited and confined to cohorts of women (65-70).

Most studies examining the impact of serum sex hormones have focused on the SHBG and albumin bound total molecule with few studies comparing the role of total versus free or bioavailable fractions and so the influence of sex hormone fractions on bone health remains unclear (71). It is well established that the age-related decline of both free and bioavailable fractions (accompanied by a rise in SHBG) starts as early as the 4th decade in men (72, 73). As most studies
of bone health have been undertaken in cohorts of elderly men of more than 60 years old, the contributions of these relatively early declines in free and bioavailable hormones to bone health in middle-aged men is unclear. The overall aim of publications 1-3 was to investigate the association between the sex hormones T and E₂, and the free and bio-available fractions of these hormones, as well as SHBG, with male bone health as measured by QUS of the heel, pQCT of the radius and serum markers of bone turnover in men aged 40-79 years of age.

3.1.2 Contribution to literature

3.1.2.1 Bone turnover and bone density

Serum markers of bone turnover were negatively associated with both QUS parameters at the heel and DXA-BMD₃ at the hip and spine, even after adjustment for age, weight and lifestyle factors. Several previous studies have found similar associations but did not adjust for the potential confounding effect of lifestyle (55, 57, 60, 62, 63). These findings confirm the important link between bone remodelling and bone health in men - even at weight-bearing sites where bone metabolism is under the strong influence of the mechanical effect of body weight.

3.1.2.2 The role of oestradiol

The results presented here confirmed in a population of middle aged as well as older men that E₂ is the most important sex steroid with respect to bone health. In keeping with previous studies (40, 43, 74, 75), higher levels of both total and free E₂ were associated with lower levels of the bone resorption
marker B-cTX. No association was observed however between total or free $E_2$ and P1NP. Whilst there is evidence that $E_2$ increases bone formation, this may be less important (40) and the main effect of $E_2$ may be to reduce bone resorption. In keeping with this and the results of previous studies (74, 76, 77), higher levels of total, free and bioavailable $E_2$ were associated with higher QUS parameters at the heel. The association was observed across the entire range of $E_2$ concentrations with no evidence of a threshold effect, in accordance with some (28) but not all (47, 74, 78, 79) previous studies. There are few data examining bone geometry in adult men and this is the first multicentre study using pQCT of the radius. A positive association between $E_2$ (total, free and bioavailable) and BMD at both the distal and midshaft radial sites was observed in one of the study centres, where there was no evidence of a threshold effect. This is in contrast to a previous study by Khosla et al. who reported an association with bioavailable $E_2$ below a threshold of 30pM at several skeletal sites including the femoral neck, distal radius and distal tibia (48). In the current study, the association with $E_2$ was stronger in the older (>60 years) men which would be consistent with a lower level of bioavailable hormone in older age. Associations were also observed between $E_2$ and cortical thickness / medullary area at the midshaft radius.

The observation that sex steroids, and in particular $E_2$, were associated with bone measurements in middle aged (<60 years) as well as older men is relatively novel as most previous studies have been conducted in elderly men. In keeping with this, a small cross-sectional study that did include middle aged as well as elderly men reported an association between sex steroids and radial
BMD_a (but not hip or spine BMD_a) (48). Taken together this suggests that hormonal mechanisms to maintain bone mass act from middle age onwards.

3.1.2.3 The role of testosterone

In contrast, the publications presented in this thesis confirm the notion that T has less influence on bone health than E_2. Total, free and bioavailable T were not associated with either bone formation or resorption. However, whilst total T was not linearly associated with QUS parameters at the heel, although men with hypogonadism (defined at total T less than 8 or 10 nmol/L) had slightly lower QUS values - a result that was weakly statistically significant and consistent with a report from the MrOS study showing reduced femoral neck BMD_a (assessed by DXA) in men with total T < 7 nmol/L (80). Weak positive associations were also observed in the EMAS men between QUS parameters and free and bioavailable T. T did not appear to have a large influence on bone geometry either. There was evidence of an association between higher T and larger total area at the distal, but not the midshaft, radius in the older (<60 years) men in one of the EMAS centres. This is in keeping with data from both adolescents and animal models suggesting that T promotes periosteal apposition leading to an increase in total bone area. However, other studies in adult men are conflicting. A study by Khosla et al. reported that higher T was associated with smaller bone area (48) whilst a DXA-based study observed no association between T and an age-related increase in periosteal apposition (49, 81).
3.1.2.4 The role of SHBG

Higher SHBG levels were associated with higher levels of both bone turnover markers, in keeping with a study in ageing women (82). This is in line with previous reports stating that SHBG is negatively correlated with BMD (83-85). Similarly, higher SHBG levels were associated with lower QUS parameters at the heel, in keeping with some (78, 84, 86, 87) but not all previous studies (46, 74). These results are perhaps unsurprising given that SHBG is involved in the transportation of gonadal steroids in serum. However, the associations with SHBG persisted after further adjustment for sex steroids, suggesting that SHBG may affect bone directly. In accordance with this, a study of older men and postmenopausal women reported that SHBG was associated with the risk of hip fracture independently of total and bioavailable E₂ and T levels (88).
3.2 Influence of vitamin D on bone health in men: publication 4

This section summarises the background, aims and contribution to the literature of publication 4. The publication focuses on the role of vitamin D in influencing bone health.

3.2.1 Background and aims

Vitamin D deficiency is common, especially in the housebound and elderly. It is assessed by measuring the most common circulating metabolite, 25-hydroxyvitamin D (25(OH)D), however strict diagnosis criteria have proved problematic to establish, in part because of natural variations in 25(OH)D levels between different populations and disease groups and also differences in assay methods (89). 1,25-dihydroxyvitamin D (1,25(OH)₂D) is derived from 25(OH)D by 1α-hydroxylation primarily in the kidney and is the metabolically active molecule responsible for most of the actions of vitamin D (90). Whilst there is an abundance of population-based data examining the association between 25-hydroxyvitamin D (25(OH)D) and bone health, even in men (89, 91-100), the influence of 1,25(OH)₂D on bone health is less well understood. 1,25(OH)₂D is present in much lower concentrations compared to 25(OH)D and it’s half life is much shorter making it difficult to measure accurately (101). Recent advances in mass spectrometry (MS) have provided more accurate measurements of many metabolic hormones, but to date very few MS-based assays for 1,25(OH)₂D have been developed. Among the few population-based studies that have measured both vitamin D metabolites and also parathyroid hormone (PTH), some provide evidence of a positive association between 1,25(OH)₂D and 25(OH)D (102-104) and between 1,25(OH)₂D and PTH (102, 103). There are very few studies
examining the influence of 1,25(OH)\(_2\)D on bone health and the data are conflicting. One study showed a doubling of the risk of hip fracture in postmenopausal women with low serum 1,25(OH)\(_2\)D (105) and another study found lower 1,25(OH)\(_2\)D levels in hip fracture patients compared to controls (106). In contrast, a small study of healthy men aged 30-92 years found no association between 1,25(OH)\(_2\)D and radial or vertebral bone mineral content (107).

The aim of the analysis presented in publication 4 was to examine the inter-relationships between 1,25(OH)\(_2\)D, 25(OH)D and PTH. The secondary aim was to examine the influence of 1,25(OH)\(_2\)D, 25(OH)D and PTH on bone health measured using quantitative ultrasound (QUS) of the heel, dual energy x-ray absorptiometry (DXA) of the hip and lumbar spine, and serum markers of bone turnover.

3.2.2 Contribution to literature
This was one of the first epidemiological studies to develop and use a state of the art MS-based assay for 1,25(OH)\(_2\)D and is the first population-based study to show that, although 1,25(OH)\(_2\)D and 25(OH)D were positively correlated, high levels of 1,25(OH)\(_2\)D were associated with poorer bone health in a well characterized group of middle aged and older men. Men in the highest tertile of 1,25(OH)\(_2\)D and lowest tertile of 25(OH)D had a lumbar spine BMD\(_a\) almost one SD lower which could equate to a two-fold increase in the risk of fracture (108).
3.2.2.1 Association with age, season of measurement and centre

1,25(OH)₂D (cross-sectionally) declined slightly with age in keeping with some (102-104) but not all studies (107, 109). Seasonal variation was observed in 1,25(OH)₂D levels, with highest levels in the summer and lowest in winter, tracking the expected seasonal variation observed in 25(OH)D levels, implying that 1,25(OH)₂D levels are also under the influence of sunlight exposure. Whilst seasonality in 25(OH)D is well established (110, 111), the observation of similar variation in 1,25(OH)₂D is reported in some previous studies (103, 104, 109, 112), while others suggest it only occurs in subjects with low 25(OH)D levels (89, 112). The seasonal variation observed in this study persisted across the range of 25(OH)D levels.

3.2.2.2 Interrelationship between 1,25(OH)₂D, 25(OH)D and PTH

The current study observed a positive correlation between 25(OH)D and 1,25(OH)₂D, consistent with the notion that 25(OH)D is the substrate for synthesis of 1,25(OH)₂D. This is in keeping with several other studies (102-104). Interestingly, only 12% of the variation in 1,25(OH)₂D was explained by 25(OH)D. In addition, whilst marked centre differences in both 1,25(OH)₂D and 25(OH)D were observed, these differences were unrelated to latitude and there were no specific patterns (i.e. some centres had low 25(OH)D and high 1,25(OH)₂D while others had the reverse). Taken together, this implies that many other factors have an influence on 1,25(OH)₂D levels. Such factors could include diet, serum calcium and phosphate, lack of physical activity / mobility, renal function and genetics (101).
The association between 1,25(OH)$_2$D and PTH observed in this study was relatively weak and was attenuated when adjustment was made for age and centre. The previous literature is inconsistent, some observing a negative correlation (102, 103) while others not (104). It is worth commenting on the lack of a strong association between 1,25(OH)$_2$D and PTH as the level of 25(OH)D below which PTH is seen to rise is commonly used to define a clinically important threshold level of serum 25(OH)D. The current study observed, as expected, a negative association between 25(OH)D and PTH in agreement with several previous studies (96, 113, 114).

3.2.2.3 Influence of 1,25(OH)$_2$D on bone health

This is the first population-based study to examine the association between 1,25(OH)$_2$D and serum markers of bone turnover and QUS at the heel. 1,25(OH)$_2$D was positively associated with the bone resorption marker β-cTX, after adjustment for a number of potential confounding factors: age, centre, height, weight, lifestyle factors (PASE score, current smoking and alcohol consumption) and season of measurement. There was no association with the bone formation marker P1NP. 1,25(OH)$_2$D levels were associated with bone density after similar adjustments, though the strongest associations were observed at the highest levels of 1,25(OH)$_2$D. Compared to those in the lowest quintile of 1,25(OH)$_2$D, those in the highest quintile (<72.2 pg/mL) had significantly lower QUS parameters and DXA BMD$_a$ at the hip and lumbar spine. This suggests that 1,25(OH)$_2$D (particularly at high levels) may decrease bone density by increasing bone resorption and there is evidence from in-vitro and animal studies to support this (115, 116). However, there is little
epidemiological data examining the influence of 1,25(OH)₂D on bone health and the results are conflicting. The Study of Osteoporotic Fractures (SOF) Research Group reported a doubling in the risk of hip fracture in postmenopausal women with a 1,25(OH)₂D level of less than or equal to 23 pg/mL (55 pmol/L) (105). In contrast, two studies found no relationship between 1,25(OH)₂D and either radial or vertebral bone mineral content (107), or hip fracture (106).

3.2.2.4 Influence of 25(OH)D on bone health

In the current study, there was no association between 25(OH)D and either of the markers of bone turnover, in keeping with a report from the MINOS study (100), but in contrast to a Dutch study of older men and women (96). However, there were significant associations between higher 25(OH)D and greater bone density as measured by QUS and DXA. There was some inconsistency across the categories when 25(OH)D was categorized into quintiles, but no evidence of any threshold effects. These results are consistent with many previous studies (91-100). However, given that 25(OH)D is also an excellent marker of general health, it is difficult to draw any firm conclusions about causality.

3.2.2.5 Influence of PTH on bone health

Higher levels of PTH were associated with higher levels of both bone turnover markers, a relationship that appeared to be linear. There is few data examining the association between PTH and markers of bone turnover, but the results in publication 4 are consistent with a report from the community-based MINOS study of men aged 55-85 years (100). PTH was unrelated to the QUS parameters
but was weakly associated with BMD$_a$ at the total hip. This is in agreement with some other community based studies of men (97, 98, 100, 117).
3.3 Influence of lifestyle factors on bone health in men: publication 5

This section summarises the background, aims and contribution to the literature of publication 5. This publication focuses on the role of lifestyle factors on bone health.

3.3.1 Background and aims

QUS measurements at the heel have been shown to be associated with risk of fracture in men and women (118-126). Reports suggest that the strength of the prediction is as strong as that for BMDa measurements assessed using DXA. The determinants of BMDa at the hip and lumbar spine as measured by DXA, and in particular the influence of lifestyle factors such as physical activity and smoking, have received plenty of attention. Less is known about the influence of lifestyle factors on heel ultrasound parameters. There is some evidence that physical activity and smoking may influence QUS parameters in men, however, the findings have not always been consistent (127-135). The aim of the publication was to explore the associations between lifestyle factors and QUS parameters at the heel in middle aged and older men.

3.3.2 Contribution to literature

The key findings from this publication were that lifestyle factors influence bone health as assessed by heel QUS in middle aged as well as older men. Higher levels of physical activity, whether measured by self report or by direct measure of physical performance, were associated with higher QUS parameters. Smoking was linked to lower QUS while a U-shaped relationship with alcohol consumption was observed: compared to men who drank alcohol moderately (1-
men who drank no alcohol and men who drank alcohol at least 3 days per week had lower QUS parameters. These results persisted after adjustment for age, centre and weight. How does the magnitude of the results presented here equate to fracture risk? Data from a large prospective study showed that the risk of both hip and non hip fracture increased by a factor of two fold for each unit (SD) change in broadband ultrasound attenuation (BUA) and speed of sound (SOS) (122). In this study the difference in SOS between those who did and did not smoke equated to around a third of a standard deviation of the measurement, while for the physical activity scores (PASE and SF36 physical component score) the difference in SOS between those in the lowest and highest tertiles of activity was about one fifth of an SD. Although the risk of fracture attributable to the different exposures is relatively small, given that they are potentially modifiable and common they would certainly be potential candidates for inclusion in a population strategy for fracture prevention to optimise bone health in middle age and elderly men with the ultimate aim of reducing fracture occurrence.

3.3.2.1 Influence of age

The average percent annual decline observed in this study was 0.16% for BUA, 0.03% for SOS and 0.23% for quantitative ultrasound index (QUI). In an observational study of 1,138 Spanish men aged 18-99, using the same measurement device, the QUS parameters declined by between 0.08 and 0.41% per year (136). In keeping with our finding, a decrease in QUS with age has been observed in other studies which used other sonometers (127, 135, 137-140).
3.3.2.2 Influence of physical activity

In this study physical activity was positively associated with the QUS parameters. Most, though not all, previous studies demonstrate a positive impact of physical activity on heel ultrasound parameters in men (127-135). In one of the largest studies of 4981 men, aged 60-80 years, recalled physical activity was linked with increased QUI as measured using a Lunar Achilles device (127). Most studies have focused on historical or self report of physical activity however in this analysis an association was also observed with two physical performance measures, including the time taken to sit and stand five times, and the time to walk 50 feet; those who took longer to perform these activities having lower ultrasound parameters. It seems likely that these are more like measures of function, proxies for higher intensity activities and consequent bone loading although it is not possible to confirm or refute this (141). Also our assessment of bone health was at the heel and it is possible that the effect may differ at other skeletal sites.

3.3.2.3 Influence of smoking and alcohol consumption

In this study there was an association between smoking, both ever and current and lower QUS measures. Previous studies that examine the link between QUS and smoking using both the Sahara device and other sonometers provide somewhat discrepant findings, with some though not all reporting a negative association (127-129, 133-135, 139, 140, 142). A study in an older German population published after the paper presented in this thesis also confirms the negative impact of smoking (143). There are fewer data concerning the impact of alcohol consumption with most suggesting no association (127, 128, 133, 144-
In this study there was a U-shaped association between alcohol consumption and the QUS parameters, where compared to moderate drinking, both light and heavy drinking was associated with a reduction in QUS. This is consistent with some data from bone mass measurement studies finding that social drinking is associated with beneficial effects on bone mass (149). Since publication of this work, a population based study of just over 500 Australian men aged 65 years and over observed that higher alcohol consumption (assessed via a food frequency questionnaire) was associated with lower QUS parameters at the heel (150). The fact that the literature continues to be discrepant suggests that the true influence of alcohol consumption on bone health (i.e. is it causally related or just a reflection of the fact that alcohol consumption is a marker of general health) remains to be determined.
3.4 Influence of childhood fracture on BMD & future fracture: publication 6

This section summarises the background, aims and contribution to the literature of publication 6. This publication examines whether fractures occurring in childhood increase the risk of fractures later in life.

3.4.1 Background and aims

The observation that peak bone mass is attained by the end of adolescence (151) has given rise to increasing interest in potential determinants of osteoporosis that occur in early life. Factors that have a negative impact on peak bone mass are of interest in that they may have a significant impact on the risk of fragility fractures in later life.

Over 40% of girls and 50% of boys sustain at least one fracture during childhood and adolescence (152). Several studies have demonstrated an association between lower bone density or weaker bone structure and distal forearm fracture in children (153-157). A review of case-control studies and a prospective study also report an association between low BMD and fractures in childhood (158, 159).

There is good evidence that fractures in adulthood are associated with an increase in risk of a future fracture (160, 161). There is some evidence that peri / pre-menopausal fractures in women are associated with an increased risk and in one study fractures as early as age 20 were associated with risk of future fracture (162-166). There is though no data examining the impact of childhood fracture on the risk of fracture in later life. Such data are important as it potentially provides an important rationale for considering affected individuals
for assessment and therapy to prevent further bone loss and reduce morbidity in later life.

The aim of this analysis was to determine whether fractures reported in childhood were linked with lower BMD and an increased risk of fractures in later adult life.

3.4.2 Contribution to literature
At the time of publication, this was the only study examining the impact of childhood fracture on the occurrence of fracture in later life in men and women. This perhaps reflects the difficulties in conducting such a study. As the majority of fractures occur after the age of 60, there is potentially a long time between the events in childhood and any subsequent fractures in adulthood. Nevertheless, this study would suggest that a recalled history of fracture during childhood is not an important determinant of fracture risk in older men and women. However given the limitations of using recalled childhood fracture information, further studies are needed to determine if a history of fracture in childhood should be included in the assessment of risk of future fracture.
In the EPOS study, femoral neck and lumbar spine BMD was similar in those who did and did not report a fracture in childhood. This was true in both men and women and is in keeping with the findings relating to fracture in later life. This was true also when the analysis was restricted to those with a childhood forearm fracture. We are unaware of any other studies that have examined the influence of childhood fracture on BMD in adulthood.
A possible physiological explanation for the lack of association between fractures in childhood and BMD / future fractures in adulthood could be that
the skeletal envelope fills in late adolescence / early adulthood. Hence observations that fractures are associated with lower bone mass in children (153, 158, 159) and that peak fracture occurrence in childhood coincides with the maximum dissociation between increase in height and mineralisation of the skeleton (167, 168), may not impact upon fracture risk in adult life. However, a recent study by Buttozzoni et al. (169) observed persistent defects in bone density and structure in men who reported a fracture in childhood compared to control men who did not report a childhood fracture. The bone size of the men at the radius and tibia was also smaller compared to the controls, potentially reducing bone strength.
Since publication of the paper, a more recent population-based study published in 2013 including 1086 boys and 690 girls aged ≤ 18 years who could be followed up after age 35 using linked medical records, reported that distal forearm fracture in boys was associated with an increase in risk of fragility fracture in adulthood (170). This is in contrast to the EPOS results described here suggesting that fractures in childhood do not increase the risk of future fracture or vertebral deformity. It was suggested that the difference in result could be due to ascertainment bias (170) given that the EPOS study observed recalled childhood fractures in 8.9% of men and 4.5% of women, lower than in other studies, including the longitudinal Dunedin Multidisciplinary Health and Development Study, which observed fractures in 23.4% of boys and 15.8% of girls (152). Another possible explanation may be related to the age at which the childhood fracture occurred. The EPOS study included fractures occurring between the ages of 8 and 18 years of age while the 2013 study of Amin et al. included fractures at all ages (170). The majority of childhood fractures,
however, occur after the age of 8 and so this difference in inclusion criteria is unlikely to have had a large effect on the results. Despite these differences in study design and analysis, Amin et al. did not observe an association between childhood fracture and future fracture in girls in keeping with the results presented here and another study of 90 postmenopausal women (171).
3.5 Implications for future research

The results presented in this thesis were based on cross-sectional information and need to be confirmed in longitudinal data to allow some of the temporal relationships to be determined. Such studies would also be able to examine change (in both bone health and risk factors such as sex hormones) within an individual in addition to between individuals. The associations between bone health and factors such as hormones, vitamin D and lifestyle factors were observed in middle aged men under the age of 60 in addition to men aged 60+. Future work should not just focus on the elderly and include middle aged men to better elucidate if hormonal and lifestyle mechanisms to maintain bone mass act from middle age onwards. Given some of the differences observed between total T and free (and bioavailable) T, further work is needed - perhaps by measuring the bioavailable fractions directly - to understand their precise role in maintaining bone health. The persistent and often independent effect of SHBG on bone health reported here was interesting and further work is required to determine the precise physiological role of SHBG on bone health.

The results reported in this thesis offered a possible glimpse of the impact of sex hormones on bone density and geometry as measured by pQCT. However, the inconsistency of the results limited their impact. Longitudinal studies using technologies using pQCT are needed to better understand the impact of hormones on bone geometry. The unexpected observation that higher levels of 1,25(OH)₂D were associated with worse bone health was very interesting and merits further investigation. The seasonal variation observed in 1,25(OH)₂D - mapping to the expected seasonal variation in 25(OH)D - requires replication. Further work is required examining the role of the fractions of vitamin D, its
transportation via vitamin D binding protein and also the interaction with the vitamin D receptor would give us a better understanding of the physiology of vitamin D. With respect to the impact of lifestyle factors on bone health, the U-shaped association observed here between bone health and alcohol consumption was interesting and merits further work to elucidate if this is a casual relationship or merely a marker of poor general health. Studies with more accurate and more comprehensive measurements of alcohol consumption should help. From the data reported in this thesis, it appears that fractures in childhood do not influence future fracture, but given that this was based on recalled childhood fracture data, further work is needed to confirm this. Whilst it would be difficult to conduct an observational study to address this question because of the large time frames involved, the increased availability of linked electronic health record data collected from routine clinical practice has great potential to be able to investigate the influence of long term risk factors such as childhood fracture.
Chapter 4. Scientific impact

Publications 1 and 2 were published in Osteoporosis International (the journal of the International Osteoporosis Foundation and the National Osteoporosis Foundation of the USA), the leading journal worldwide dedicated solely to osteoporosis. In 2012, it had an Impact Factor of 4.039 and was ranked 33rd out of 121 journals in the category of Endocrinology and Metabolism. Publication 3 was published in the European Journal of Endocrinology, the Journal of the European Society of Endocrinology which has an impact factor of 3.136 and was ranked 51st out of 121 journals in the category of Endocrinology and Metabolism. Publication 4 was in the Journal of Clinical Endocrinology and Metabolism; published by the Endocrine Society, it is one of the premier peer-reviewed journals for clinical endocrine research. In 2012, it had an Impact Factor of 6.430 and was ranked 13th out of 121 journals in the category of Endocrinology and Metabolism. Publication 5 was published in Calcified Tissue International; affiliated to the International Osteoporosis Foundation, it had an impact factor of 2.495 in 2012 and was ranked 65th out of 121 journals in the category of Endocrinology and Metabolism. Publication 6 was in the Journal of Bone and Mineral Research; published by the American Society for Bone and Mineral Research, it is one of the premier peer-reviewed journals in the field of bone research. In 2012, it had an Impact Factor of 6.128 and was ranked 14th out of 121 journals in the category of Endocrinology and Metabolism.

As of April 2014, the work presented in this thesis has been cited 52 times. Publication 1 has been cited 14 times (36, 172-184). Publication 2 has been cited 10 times (36, 178, 180, 181, 185-190) and publication 3 cited 5 times (36,
180, 184, 188, 191). Publication 4 has been cited 6 times (36, 192-196) and was the subject of an online article on the Endocrine Today website: http://bit.ly/XUJqDL. Publication 5 has been cited 9 times (180, 181, 197-203) and publication 6 cited 8 times (169, 170, 204-209).

Data from this thesis has been presented at departmental, national and international conferences and I was awarded a Young Investigator award by the National Osteoporosis Society for work based on publication 6.
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Publication 1: Gonadal sex steroid status and bone health in middle-aged and elderly European men
Gonadal sex steroid status and bone health in middle-aged and elderly European men

D. Vanderschueren · S. R. Pye · K. Venken · H. Borghs · J. Gaytant · I. T. Huhtaniemi · J. E. Adams · K. A. Ward · G. Bartfai · F. F. Casanueva · J. D. Finn · G. Forti · A. Giwercman · T. S. Han · K. Kula · F. Labrie · M. E. J. Lean · N. Pendleton · M. Punab · A. J. Silman · F. C. W. Wu · T. W. O’Neill · S. Boonen · The EMAS Study Group

Received: 20 March 2009 / Accepted: 3 September 2009 / Published online: 15 December 2009

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Abstract

The influence of sex steroids on calcaneal quantitative ultrasound (QUS) parameters was assessed in a population sample of middle-aged and elderly European men. Higher free and total E2 though not testosterone, were independently associated with higher QUS parameters.

Introduction

The aim of this study was to investigate the association between QUS parameters and sex steroids in middle-aged and elderly European men.

Methods

Three thousand one hundred forty-one men aged between 40 and 79 years were recruited from eight European centres for participation in a study of male ageing: the European Male Ageing Study. Subjects were
invited by letter to attend for an interviewer-administered questionnaire, blood sample and QUS of the calcaneus (Hologic-SAHARA). Blood was assessed for sex steroids including oestradiol (E2), testosterone (T), free and bioavailable E2 and T and sex hormone binding globulin (SHBG).

Results Serum total T was not associated with any of the QUS parameters. Free T and both free and total E2 were positively related to all QUS readings, while SHBG concentrations were negatively associated. These relationships were observed in both older and younger (<60 years) men. In a multivariate model, after adjustment for age, centre, height, weight, physical activity levels and smoking, free E2 and SHBG, though not free T, remained independently associated with the QUS parameters. After further adjustment for IGF-1, however, the association with SHBG became non-significant.

Conclusion Higher free and total E2 are associated with bone health not only among the elderly but also middle-aged European men.

Keywords Epidemiology · Oestradiol · Sex steroids · SHBG · Testosterone · Ultrasound

Introduction

Osteoporosis is an important clinical and public health problem in men through its association with age-related fractures which account for substantial morbidity, economic cost and even mortality [1, 2]. The pathophysiology of bone loss in men is less well understood than in women. There is increasing evidence for an effect of sex steroids, and in particular oestradiol (E2), on the maintenance of bone health not only in women but also in elderly men [3–9]. The effect of testosterone (T) on bone health is less well understood. Most studies have been undertaken in cohorts of elderly men (more than 60 years old) with few data in younger men. In a recent study of community-dwelling Australian older men (60 years and older), serum testosterone was independently associated with the risk of osteoporotic fracture [10], while in a European study serum testosterone had no effect on fracture risk [11]. The relative role of age-related changes in total vs free or bio-available fractions of serum sex steroids on bone health remains unclear [12]. Serum T and E2 are bound with high and low affinity to serum sex hormone binding globulin (SHBG) and albumin, respectively. Only fractions of circulating sex steroid are either free (non-bound to SHBG and albumin) or bio-available (non-bound to SHBG) [13]. Moreover, it is well established that the age-related decline of both free and bio-available fractions of sex steroids in men as well as a rise of SHBG starts as early as the fourth decade [14, 15]. Reductions in free and bio-available fractions of sex steroids are also more important than decreases of total concentrations. However, the relative contribution of total vs free or bio-available sex steroids in maintaining bone health in large samples of elderly and especially middle-aged men has received little attention. The question therefore remains whether these relatively early reductions of free and bio-available sex steroids as well as the rise of SHBG contribute to a decline of bone health in middle-aged men.
The European Male Ageing Study (EMAS) is a population-based survey of ageing in both middle-aged and elderly European men. The aim of this study is to investigate the association between sex hormones, including T and E2, and the free and bio-available fractions of these hormones as well as SHBG with male bone health as estimated by quantitative ultrasound (QUS) of the calcaneus from 40 up to 80 years of age.

Materials and methods

Subjects

The subjects included in this analysis were recruited for participation in EMAS. Details concerning the study design and recruitment were described previously [16]. Briefly, men were recruited from population-based sampling frames in eight centres: Florence (Italy), Leuven (Belgium), Lodz (Poland), Malmö (Sweden), Manchester (UK), Santiago de Compostela (Spain), Szeged (Hungary) and Tartu (Estonia). Participating centres were selected to provide geographical and socioeconomic diversity within Europe and facilities to perform epidemiological surveys. Stratified random sampling was used with the aim of recruiting equal numbers of men in each of four 10-year age bands: 40–49, 50–59, 60–69 and 70 years and over. Subjects were invited by letter to complete a postal questionnaire and attend for an interviewer-assisted questionnaire, which included questions about physical activity, and QUS of the heel. Subjects were recontacted usually within 4 weeks if they did not reply following a first letter.

Study questionnaires and clinical data

The postal questionnaire included questions concerning current smoking and alcohol consumption in the previous year (response set = every day/5–6 days per week/3–4 days per week/1–2 days per week/less than once a week/not at all). There was a question about prior fracture since the age of 25 years (response set = no/yes/don’t know). The main study questionnaire included the physical activity scale for the elderly (PASE). This survey is designed to assess physical activity in epidemiologic studies of elderly persons. The PASE score combines information on leisure, household and occupational activity and is a continuous scale ranging from 0 to 1,100 [17]. There was a question about medications that subjects were taking. Height and weight were measured in a standardised fashion. Body weight was measured to the nearest 0.1 kg using an electronic scale (SECA, model no. 8801321009, SECA UK Ltd) and height to the nearest 1 mm using a stadiometer (Leicester Height Measure, SECA UK Ltd). Each centre’s electronic scales and stadiometers were calibrated on a monthly basis.

Hormone measurements

A single fasting morning (before 1000 hours) venous blood sample was obtained from all subjects. Serum was separated immediately after phlebotomy and stored at −80°C until assay at the end of the baseline study. Measurement of T and E2 were carried out by gas chromatography mass spectrometry as described in Labrie et al. [18, 19]. The lower limit of T quantitation was 0.17 nmol/L and E2 was 7.34 pmol/L. The coefficients of variation of T measurements were 2.9% within runs and 3.4% between runs and for E2 were 3.5% within runs and 3.7% between runs. SHBG was measured by the Modular E170 platform electrochemiluminescence immunoassay (Roche Diagnostics, Mannheim, Germany) as previously described [20]. The free and bio-available (non-SHBG-bound) T and E2 levels were derived from total hormone, SHBG and albumin concentrations using mass action equations and associations constants of Vermeulen et al. [21] and Van Pottelbergh et al. [22]. In addition, samples were transported in frozen state to a single laboratory for measurement of IGF-1 (University of Santiago de Compostela) by quimioluminescence. Within- and between-assay coefficients of variation (CVs) for IGF-1 were 7.4% and 2.9%, respectively. The detection limit of the assay was 20 ng/ml.

Quantitative ultrasound of the heel

Quantitative ultrasound of the left heel was performed with the Sahara Clinical Sonometer (Hologic, Bedford, MA) using a standardised protocol. Each centre used the same machine model, and each was calibrated daily with the physical phantom provided by the manufacturer. Outputs included the rate of loss of ultrasonic intensity with frequency (broadband ultrasound attenuation [BUA]) measured in dB/MHz using Fourier transformation of the recorded signal) and the velocity of ultrasound transmission through bone (speed of sound [SOS] measured in metres per second from the sound propagation time between the transducers). In addition, quantitative ultrasound index (QUI), a measure of stiffness, was derived from the BUA and SOS measures: QUI=0.41(SOS)+0.41(BUA)+571. Short-term precision of the method was established on duplicate measurements performed in 20 randomly selected cohort members in Leuven. The in vivo CVs were 2.8% and 0.3% for BUA and SOS, respectively, and 2.3% for QUI. Repeat measurements (ten) were performed on a roving phantom at each of the eight centres. Standardised
CVs (SCVs) for within-machine variability ranged by centre: for SOS, from 1.0% to 5.6%, and BUA, from 0.7% to 2.7%. SCVs for between-machines variability were 4.8% for BUA and 9.7% for SOS [23].

Analysis

Descriptive statistics were used to characterise the distribution of the heel QUS parameters (BUA, SOS and QUI) and sex hormone levels (after exclusion of 144 subjects who were taking therapies which may have influenced sex steroid levels). Linear regression was used to determine the association between each of the ultrasound parameters and the different sex steroid levels adjusting for age, height, weight, physical activity, smoking and centre with the results expressed as β coefficients and 95% confidence intervals (CI). The sex hormone variables were analysed as continuous data and categorised into quintiles, though for total T we also categorised individuals as either normal or hypogonadal (using two thresholds, 8 and 10 nmol/L) [24]. We examined initially the whole group and subsequently after stratification by age: less than and greater than 60 years. The associations were assessed visually using scatter plots, super-imposing linear lines and locally weighted scatter plot smooth (lowess) curves [25]. Statistical analysis was performed using STATA version 9.2 (http://www.stata.com).

Results

Subjects

Three thousand one hundred forty-one men (mean age 59.7 years) were included in the analysis. Of these, 1,618 were less than 60 years, and 1,523 were 60 years or older. Mean height was 173.7 cm and weight 83.5 kg; see Table 1. Mean PASE score was 198.1 (SD=91.4). Of the men, 21% reported that they currently smoke, whilst 57% reported consuming alcohol at least 1 day per week. Twenty-six per cent reported a previous fracture since the age of 25. Mean BUA was 80.2 dB/MHz (SD=18.7) and SOS 1,550.9 m/s (SD=33.7). QUI was 97.9 (SD=21.5). The QUS parameters were all associated with prior fracture: after adjustment for age and centre, compared to those who did not report a previous fracture, those who did had a lower BUA (β coefficient=−4.797 dB/MHz, p<0.001), SOS (β coefficient=−9.491 m/s, p<0.001) and QUI (β coefficient=−5.860, p<0.001).

The mean values for the sex hormones are shown in Table 2. Mean T level was 16.6 nmol/L, free T 292.2 pmol/L, and bio-available T was 7.1 nmol/L. Mean E2 level was 74.0 pmol/L, free E2 1.3 pmol/L, bio-available E2 51.2 pmol/L, SHBG 42.7 nmol/L and IGF-1 133.2 ng/mL. Using a cut-off of serum total T concentration of 8 and 10 nmol/L, respectively, 4.1% and 11.6% of men respectively had evidence of T deficiency.

Influence of sex hormones on ultrasound parameters

After adjustment for age, centre, height, weight and physical activity as determined by PASE score and current smoking, there were significant positive associations between the QUS parameters BUA, SOS and QUI and serum gonadal steroid concentrations including both free, total and bio-available E2 and free and bio-available T; see Table 3. No association with total T was present, though the QUS parameters were significantly lower in hypogonadal

### Table 1 Subject characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>N=3,141</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (SD)</td>
<td></td>
</tr>
<tr>
<td>Age at interview (years)</td>
<td>59.7 (10.9)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>173.7 (7.3)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>83.5 (13.8)</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>27.6 (4.0)</td>
</tr>
<tr>
<td>PASE score (0–1,100)</td>
<td>198.1 (91.4)</td>
</tr>
<tr>
<td>Broadband ultrasound attenuation (dB/MHz)</td>
<td>80.2 (18.7)</td>
</tr>
<tr>
<td>Speed of sound (m/s)</td>
<td>1,550.9 (33.7)</td>
</tr>
<tr>
<td>Quantitative ultrasound index</td>
<td>97.9 (21.5)</td>
</tr>
</tbody>
</table>

| %                             |         |
| Currently smoke (yes vs no)   | 21.2 |
| Alcohol consumption ≥1 day/week | 56.7 |
| Previous fracture since age 25 (yes vs no) | 25.9 |

### Table 2 Sex hormone descriptives

<table>
<thead>
<tr>
<th>Variable</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Mean (SD)</td>
<td></td>
</tr>
<tr>
<td>Testosterone (nmol/L)</td>
<td>16.6 (5.9)</td>
</tr>
<tr>
<td>Free testosterone (pmol/L)</td>
<td>292.2 (87.2)</td>
</tr>
<tr>
<td>Bio-available testosterone (nmol/L)</td>
<td>7.1 (2.2)</td>
</tr>
<tr>
<td>Oestradiol (pmol/L)</td>
<td>74.0 (25.0)</td>
</tr>
<tr>
<td>Free oestradiol (pmol/L)</td>
<td>1.3 (0.4)</td>
</tr>
<tr>
<td>Bio-available oestradiol (pmol/L)</td>
<td>51.2 (17.3)</td>
</tr>
<tr>
<td>SHBG (nmol/L)</td>
<td>42.7 (19.6)</td>
</tr>
<tr>
<td>IGF-1 (ng/mL)</td>
<td>133.2 (43.4)</td>
</tr>
</tbody>
</table>

| N (%)                         |         |
| Testosterone level<8 nmol/L   | 128 (4.1) |
| Testosterone level<10 nmol/L  | 361 (11.6) |
men compared to eugonadal men using either the 8 or 10 nmol/L threshold. In contrast with the gonadal sex steroid concentrations, SHBG levels were negatively associated with all three QUS parameters. After stratification by age, broadly similar associations were observed above and below the age of 60 years for total, free and bio E\(_2\) levels, though stronger associations were observed with free and bio T levels among the older men (data not shown). Figure 1 shows the relationship between free E\(_2\) levels and BUA and SOS. Using a locally weighted scatterplot smoothed line, the main effect was increased BUA and SOS with increased values of E\(_2\) with no evidence of a threshold effect. In a multivariate model including free T, free E\(_2\) and SHBG as continuous variables, after adjustment for age, centre, height, weight, physical activity (as assessed by PASE) and current smoking, both free E\(_2\) and SHBG remained significantly associated with all three QUS parameters; see Table 4. Overall, the multivariate model explained 10–12% of the overall variation of QUS measures. After further adjustment for IGF-1, the association with SHBG became non-significant (data not shown). In a multivariate model using the same variables with the sex steroids and SHBG categorised into quintiles (see also Table 4), higher free E\(_2\) levels were associated with higher QUS parameters. For SHBG, the association appeared non-linear; compared to the lowest quintile, those in the second and third quintiles had lower measurements. On formal testing, there was a significant negative (linear) trend with BUA only, though this disappeared after adjustment for IGF-1 (data not shown). Results were the same when either total or bio-available E\(_2\) were used instead of free E\(_2\) (data not shown).

### Discussion

In this study, we found no association between any of the QUS parameters and total T. There were significant associations between the QUS parameters, E\(_2\), free T and SHBG, and these were observed in men both above and below 60 years of age. As expected, the results were similar for free and bio-available sex steroid concentrations. In a multivariate analysis including free E\(_2\), SHBG and free T, the association with free T was no longer significant.

EMAS is the first study to explore the relationship between gonadal steroid status and bone health as measured by QUS of the calcaneus in a large group of both middle-aged and elderly men. This type of data is important as mechanisms related to bone loss in old age may differ from those related to maintenance of peak bone mass in middle-aged men. In ageing men, reductions in free and bio-available fractions of sex steroids which may negatively impact on bone health are already apparent from the fourth decade on. Our data are consistent with previous studies in older men showing that bone health is more strongly...
associated with (total and free) \(E_2\) than with \(T\). In fact, total \(T\) was not significantly related to any of the bone parameters in this cohort, irrespective of age. However, compared to those with normal levels of \(T\), those with hypogonadism as based on a threshold of 8 or 10 nmol/L [24, 26] had lower BUA, SOS and QUI. This is consistent with recent findings in the MrOS study in which femoral bone mineral density (BMDa) (assessed by dual-energy X-ray absorptiometry [DXA]) was reduced only in men with \(T\) levels <7 nmol/L [27].

In agreement with previous studies [28–31], consistent positive associations between \(E_2\) and all QUS parameters were found both in middle-aged and in elderly men. Our findings add further evidence to support the view that \(E_2\) is the major determinant of bone health in elderly men. In keeping with some [22] but not all studies [28, 32–34], we found a positive association across the entire range of \(E_2\) concentrations with no evidence of a threshold effect.

The fact that SHBG binds but also inactivates gonadal steroids is an important determinant of the bioavailability for tissue action and metabolism. Not surprisingly, and in accordance with most [33, 35–37] but not all earlier observations [11, 28], serum SHBG was inversely related to bone QUS parameters in the men in our study. It is well established that serum SHBG starts to rise in men as early as the fourth or fifth decade [14, 15]. The resulting age-related decrease of bioavailability of free sex steroids may explain the negative correlation of sex hormone binding globulin with bone density. An alternative explanation is that SHBG may have a direct negative effect of bone, independently of sex steroids. Interestingly, in line with our findings in men, SHBG has been reported to be associated with hip fracture risk, both in older men and postmenopausal women, independently of serum total and bioavailable \(E_2\) and \(T\) concentrations [9, 38].

That sex steroids are a determinant of bone density not only in elderly (>60 years) but also middle-aged men is a novel observation. Most previous studies have been small in scale or undertaken only in elderly men. In keeping with our finding, similar associations have been observed between sex steroid levels and bone density (at the radius but not at spine and hip) in a small cross-sectional sample of middle-aged (and elderly) men [39]. This suggests that hormonal mechanisms to maintain bone mass act from middle age onwards.

In our study, serum concentrations of sex steroids accounted only for a small proportion of the age-related decrease in QUS parameters. This underlines the fact that age-related bone loss in men (at least as determined by QUS) is due to a complex interplay of hormonal changes and other contributing factors.

The main strength of our study is that it is based on large-scale, population-based data and on the use of standardised methods to assess bone health and sex hormone status. There are a number of limitations to consider in interpreting the results. The study was cross-sectional, and given this design, it is not possible to determine the temporal nature of the observed associations for which prospective data are needed. The overall response rate for participation was 45%. It is possible that those invited but who did not take part may differ with respect to levels of sex hormones than those who took part, and therefore the data concerning absolute levels of these parameters need to be interpreted with caution. Any factors influencing participation, however, are unlikely to have influenced the association between the sex hormones and QUS parameters which are based on an internal comparison of those who participated. Our results are based on assessment of the calcaneus, a predominantly (95%)}
trabecular bone which may be more sensitive to variation in sex hormone levels than cortical bone. The results may therefore be difficult to extrapolate to other skeletal sites. Unlike DXA there are no published methods for cross-calibrating between QUS scanners, and the results reported are the data obtained at each centre (though we included an adjustment for centre in the analysis). Any errors related to measurement are, however, likely to be non-directional and would tend, if anything, to reduce the risk of finding significant biological associations. The study was based on assessment of middle-aged and elderly European men, and extrapolation beyond this group should be undertaken with caution. In addition, our findings are based on QUS of the calcaneus as a measure of bone architecture rather than DXA. Prospective studies have, however, confirmed the value of QUS for predicting fracture risk in both sexes. In ageing men, QUS measurements predict the risk of hip and any non-spine fracture and almost as well as hip BMDa measurements [40].

In conclusion, higher free and total E2 are associated with bone health not only among the elderly but also middle-aged European men. The overall effect on male bone health of these hormonal variations, however, is modest.

Acknowledgements The European Male Ageing Study is funded by the Commission of the European Communities Fifth Framework Programme “Quality of Life and Management of Living Resources” Grant QLK6-CT-2001-00258 and supported by funding from the UK Arthritis Research Campaign. For additional information regarding EMAS, contact Frederick Wu, MD; Dept of Endocrinology, Manchester Royal Infirmary, UK. The authors wish to thank the men who participated in the eight countries, the research/nursing staff in the eight centres: C Pott, Manchester, E Wouters, Leuven, M Nilsson, Malmö, M del Mar Fernandez, Santiago de Compostela, M Jedrzejowska, Lodz, H-M Tabo, Tartu, A Heredi, Szeged for their data collection and C

<p>| Table 4 Association between free testosterone, free oestradiol and sex hormone binding globulin and QUS parameters |
|---------------------------------|------------------|------------------|------------------|</p>
<table>
<thead>
<tr>
<th></th>
<th>BUA (dB/Mhz)</th>
<th>SOS (m/s)</th>
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<tr>
<td><strong>β coefficient (95% CI)</strong></td>
<td><strong>P</strong></td>
<td><strong>β coefficient (95% CI)</strong></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.050 (−0.057, 0.157)a</td>
<td>0.110 (−0.083, 0.303)a</td>
<td>0.073 (−0.050, 0.196)a</td>
<td></td>
</tr>
<tr>
<td>Free oestradiol (per 10 pmol/L)</td>
<td>37.407 (18.152, 56.662)a</td>
<td>76.184 (41.375, 110.994)a</td>
<td>46.447 (24.325, 68.569)a</td>
</tr>
<tr>
<td>SHBG (per 10 nmol/L)</td>
<td>−0.752 (−1.137, −0.367)a</td>
<td>−0.910 (−1.606, −0.213)a</td>
<td>−0.581 (−1.020, −0.141)a</td>
</tr>
<tr>
<td>Free testosterone quintiles (pmol/L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;221</td>
<td>Referent b</td>
<td>Referent b</td>
<td>Referent b</td>
</tr>
<tr>
<td>221–263</td>
<td>−0.037 (−2.157, 2.084)b</td>
<td>0.528 (−3.301, 4.358)b</td>
<td>0.195 (−2.250, 2.641)b</td>
</tr>
<tr>
<td>263–307</td>
<td>1.006 (−1.232, 3.244)b</td>
<td>1.799 (−2.243, 5.841)b</td>
<td>1.114 (−1.470, 3.698)b</td>
</tr>
<tr>
<td>307–359</td>
<td>0.542 (−1.838, 2.922)b</td>
<td>1.404 (−2.895, 5.703)b</td>
<td>0.766 (−1.978, 3.510)b</td>
</tr>
<tr>
<td>≥359</td>
<td>0.192 (−2.545, 2.928)b</td>
<td>0.509b</td>
<td>0.957 (−3.986, 5.900)b</td>
</tr>
<tr>
<td>Free oestradiol quintiles (pmol/L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;0.9</td>
<td>Referent b</td>
<td>Referent b</td>
<td>Referent b</td>
</tr>
<tr>
<td>0.9–1.1</td>
<td>1.503 (−0.616, 3.622)b</td>
<td>3.390 (−0.438, 7.218)b</td>
<td>1.685 (−0.761, 4.131)b</td>
</tr>
<tr>
<td>1.1–1.3</td>
<td>3.367 (1.177, 5.557)b</td>
<td>7.047 (3.091, 11.002)b</td>
<td>3.957 (1.432, 6.481)b</td>
</tr>
<tr>
<td>1.3–1.6</td>
<td>3.074 (0.787, 5.362)b</td>
<td>6.714 (2.582, 10.846)b</td>
<td>3.764 (1.128, 6.400)b</td>
</tr>
<tr>
<td>≥1.6</td>
<td>5.659 (3.153, 8.165)b</td>
<td>11.189 (6.662, 15.715)b</td>
<td>6.563 (3.678, 9.447)b</td>
</tr>
<tr>
<td>SHBG quintiles (nmol/L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;27</td>
<td>Referent b</td>
<td>Referent b</td>
<td>Referent b</td>
</tr>
<tr>
<td>27–35</td>
<td>1.715 (−0.369, 3.799)b</td>
<td>3.296 (−0.468, 7.060)b</td>
<td>1.657 (−0.748, 4.062)b</td>
</tr>
<tr>
<td>35–43</td>
<td>1.084 (−1.079, 3.246)b</td>
<td>3.240 (−0.666, 7.146)b</td>
<td>1.563 (−0.931, 4.058)b</td>
</tr>
<tr>
<td>43–56</td>
<td>−0.040 (−2.269, 2.189)b</td>
<td>0.355 (−3.671, 4.381)b</td>
<td>0.386 (−2.185, 2.958)b</td>
</tr>
<tr>
<td>≥56</td>
<td>−2.145 (−4.510, 0.220)b</td>
<td>0.020b</td>
<td>−2.397 (−6.669, 1.875)b</td>
</tr>
</tbody>
</table>

*p<0.05

*a Multivariable model including free T, free E2 and SHBG as continuous variables; age, centre, height, weight, PASE score and current smoking

*b Multivariable model including free T, free E2 and SHBG categorised into quintiles; age, centre, height, weight, PASE score and current smoking
Moseley, Manchester for data entry and project coordination. Dr. Vanderschueren and Dr. Boonen are senior clinical investigators of the Fund for Scientific Research-Flanders, Belgium (F.W.O.-Vlaanderen). Dr. Boonen is holder of the Leuven University Chair in Metabolic Bone Diseases. K Venken is a postdoctoral fellow of the Fund for Scientific Research-Flanders (F.W.O.-Vlaanderen).

Conflicts of interest None.

References

Positive associations with serum estrogens and negative associations with androgens. J Clin Invest 100:1755–1759


Publication 2: Influence of age and sex steroids on bone density and geometry in middle-aged and elderly European men
Influence of age and sex steroids on bone density and geometry in middle-aged and elderly European men

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Received: 10 February 2010 / Accepted: 27 July 2010 / Published online: 30 October 2010
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Abstract
Summary The influence of age and sex steroids on bone density and geometry of the radius was examined in two European Caucasian populations. Age-related change in bone density and geometry was observed. In older men, bioavailable oestradiol may play a role in the maintenance of cortical and trabecular bone mineral density (BMD).

Introduction To examine the effect of age and sex steroids on bone density and geometry of the radius in two European Caucasian populations.
Methods European Caucasian men aged 40–79 years were recruited from population registers in two centres: Manchester (UK) and Leuven (Belgium), for participation in the European Male Ageing Study. Total testosterone (T) and oestradiol (E₂) were measured by mass spectrometry and the free and bioavailable fractions calculated. Peripheral quantitative computed tomography was used to scan the radius at distal (4%) and midshaft (50%) sites.

Results Three hundred thirty-nine men from Manchester and 389 from Leuven, mean ages 60.2 and 60.0 years, respectively, participated. At the 50% radius site, there was a significant decrease with age in cortical BMD, bone mineral content (BMC), cortical thickness, and muscle area, whilst medullary area increased. At the 4% radius site, trabecular and total volumetric BMD declined with age. Increasing bioavailable E₂ (bioE₂) was associated with increased cortical BMD (50% radius site) and trabecular BMD (4% radius site) in Leuven, but not Manchester, men. This effect was predominantly in those aged 60 years and over. In older Leuven men, bioavailable testosterone (Bio T) was linked with increased cortical BMC, muscle area and SSI (50% radius site) and total area (4% radius site).

Conclusions There is age-related change in bone density and geometry at the midshaft radius in middle-aged and elderly European men. In older men bioE₂ may maintain cortical and trabecular BMD. BioT may influence bone health through associations with muscle mass and bone area.

Keywords Ageing · Epidemiology · Osteoporosis · Peripheral quantitative computed tomography · Sex hormones

Introduction

Osteoporosis in men is an increasing but under-appreciated clinical and public health problem with the lifetime risk of fracture in men at age 50 years estimated at 21% [1]. As in women, increasing age is one of the major determinants of osteoporosis and fracture risk in men. Most studies examining changes in bone health with age have focused on "areal" bone mineral density (g/cm²; BMDa) [2] as measured by dual-energy X-ray absorptiometry (DXA) [3–6]. There are limitations, however, in assessment of bone health using DXA. In particular, DXA tends to overestimate BMD in larger, and underestimate in smaller, bones. Furthermore, bone strength and susceptibility to fracture is influenced not only by the bone mineral content (BMC) but also bone shape and mineral distribution and the loading conditions to which the bone is subjected. Peripheral quantitative computed tomography (pQCT) allows assessment of both bone geometry and material properties including volumetric density (BMD). In contrast to age-related changes in DXA BMDa in men there are relatively few data concerning...
change in BMD as assessed by pQCT and bone structure with age.

Levels of sex steroids are known to be associated with BMD, as assessed using DXA, and also rate of bone loss [7–13]. The contribution of oestradiol (E2) to BMD has been reasonably well established but the effect of testosterone (T) is less clear, as are the effects of sex hormones on bone structural parameters. Khosla et al. [9, 14] showed that oestradiol (E2) was the most constant predictor of BMD and geometry, measured by QCT, with the effect being more marked in elderly men as age-related declines in sex steroids become relevant. Similarly in the MINOS cohort, E2 was related to DXA BMDa cortical thickness and area [15]. There is some evidence to suggest a threshold effect of oestrogen, particularly in cortical bone, below which the male skeleton may suffer oestrogen-related bone loss similar to that in the post menopausal female—the threshold level being the median value of bioavailable (bio) E2 (<30 pM) in older (>60 years) men [8, 14]. Testosterone (T) has been linked with cortical and trabecular BMD [14, 16] with conflicting data on effects on bone geometry. Some studies have observed an association between testosterone and bone loss in males [13] whilst others have shown little or no effect, be it assessing BMD or increased fracture risk [15, 17–19]; geometric parameters were not reported in these studies.

The aims of this cross-sectional study were: firstly to determine the influence of age on BMD and bone structure at the radius in middle-aged and elderly European men; secondly to determine the relationship between BMD and bone structure with sex steroid levels, and thirdly to determine whether the strength of any association between bioE2 and BMD differ above and below a threshold level of bioE2 defined as the median value among older men (60 years and over).

### Materials and methods

#### Subjects

The subjects included in this analysis were recruited for participation in the European Male Ageing Study (EMAS), a prospective study of ageing in European Caucasian community-dwelling men. Detailed methods have been described previously [20]. Briefly, men were recruited from population-based sampling frames in eight centres between 2003 and 2005. Stratified random sampling was used with the aim of recruiting equal numbers of men in each of four 10-year age bands: 40–49 years, 50–59 years, 60–69 years, and 70–79 years. Letters of invitation were sent to subjects asking them to attend for health assessments by a range of health questionnaires, physical and cognitive performance tests, anthropometry and a fasting blood sample. In two centres, Manchester (UK) and Leuven (Belgium) subjects had pQCT measurements performed at the radius. Ethics approval for the study was obtained in accordance with local institutional requirements in each centre, and each participant gave written informed consent.

#### Peripheral quantitative computed tomography

Peripheral QCT measurements of the non-dominant radius were made in men recruited to the Manchester and Leuven centres using XCT-2000 scanners (Stratec, Pforzheim, Germany). At the distal (4%) site total and trabecular BMD (mg/cm³) and bone cross-sectional area (mm²) were measured (voxel size, 0.4 mm); the slice location at the 4% and 50% site was more distal in Leuven compared with Manchester; the reference line was placed at the distal border of the radial endplate in Leuven, in Manchester the line is placed to bisect the lateral border of the endplate these differences result in a scan site difference approximately 1–2 mm between centers. At the diaphysis (50% site, voxel size 0.6 mm), cortical BMD (mg/cm³), cortical BMC (mg/mm³), total, cortical and medullary areas (mm²), cortical thickness (mm), stress strain index (SSI, mm³) and muscle cross-sectional area, as a proxy for muscle strength (CSMA, mm²), were measured. SSI provides a measure of a bone’s torsional strength [21, 22].

### Table 1 Subject characteristics: by centre

<table>
<thead>
<tr>
<th>Variable</th>
<th>Manchester</th>
<th>Leuven</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>339</td>
<td>389</td>
</tr>
<tr>
<td>Age at interview (years)</td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
</tr>
<tr>
<td></td>
<td>60.2 (11.1)</td>
<td>60.0 (11.1)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>174.3 (7.2)</td>
<td>174.5 (7.1)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>83.8 (13.4)</td>
<td>82.1 (13.2)</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>27.5 (3.6)</td>
<td>26.9 (3.9)*</td>
</tr>
<tr>
<td>Midshaft radius</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cortical BMD (mg/cm³)</td>
<td>1,149.8 (39.8)</td>
<td>1,161.0 (38.0)*</td>
</tr>
<tr>
<td>Cortical BMC (mg/mm³)</td>
<td>120.5 (18.0)</td>
<td>124.0 (17.2)*</td>
</tr>
<tr>
<td>Total area (mm²)</td>
<td>149.5 (21.5)</td>
<td>150.5 (22.3)</td>
</tr>
<tr>
<td>Cortical thickness (mm)</td>
<td>3.2 (0.5)</td>
<td>3.2 (0.4)</td>
</tr>
<tr>
<td>Medullary area (mm²)</td>
<td>43.4 (17.2)</td>
<td>43.7 (18.9)</td>
</tr>
<tr>
<td>Cross-sectional muscle area (mm²)</td>
<td>3,558.3 (649.3)</td>
<td>3,744.8 (591.6)*</td>
</tr>
<tr>
<td>Stress strain index (mm³)</td>
<td>330.3 (63.4)</td>
<td>345.6 (67.1)*</td>
</tr>
<tr>
<td>Distal radius</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total density (mg/cm³)</td>
<td>436.3 (70.1)</td>
<td>361.1 (57.3)*</td>
</tr>
<tr>
<td>Total area (mm²)</td>
<td>341.2 (52.5)</td>
<td>413.1 (66.7)*</td>
</tr>
<tr>
<td>Trabecular density (mg/cm³)</td>
<td>203.9 (48.0)</td>
<td>205.5 (41.4)</td>
</tr>
</tbody>
</table>

*p<0.05
A detailed methodology for these measurements has been described previously [23]. For cross-calibration between Leuven and Manchester the European Forearm Phantom (EFP) was measured [24]; 10 repeat measurements were taken in slices 1–4. There were no differences greater than precision error for trabecular, total and cortical BMD, BMC or cortical area. Therefore no cross-calibration was performed between the two centres. These data and decisions were reviewed by Dr Klaus Engelke a CT expert from University of Erlangen, Germany and the scanner manufacturer Stratec Medizintechnik GmbH, Profzheim, Germany (Dr. Johannes Willnecker—personal communication).

The short term precision of two repeat radius measurements with repositioning in adults were: Manchester (n=22) Leuven (n=40) trabecular BMD 1.27%, 1.42%; total BMD 2.1%, 1.3%; cortical BMD 0.77%, 0.71%; cortical area 2.4%, 1.3%; muscle area 3.7%, 1.1. Manufacturer’s standard quality assurance procedures were followed in both centres.

Sex hormone measurement

A single-fasting morning (before 10.00 h) venous blood sample was obtained from all subjects. Serum was separated immediately after phlebotomy and stored at −80°C until assay at the end of the baseline study. Measurement of T and E₂ were carried out by gas chromatography mass spectrometry as described in Labrie et al. [25, 26]. The lower limit of T quantitation was 0.17 nmol/L and E₂ was 7.34 pmol/L. The coefficients of variation of T measurements were 2.9% within runs and 3.4% between runs, and for E₂, were 3.5% within runs and 3.7% between runs. SHBG was measured by the Modular E170 platform electrochemiluminescence immunoassay (Roche Diagnostics, Mannheim, Germany) as previously described [27]. Free and bioT and E₂ levels were derived from total T, total E₂, SHBG, and albumin concentrations using mass action equations and associations constants of Van Pottelbergh et al. and Vermeulen et al. [12, 28].

Table 2  Sex hormone descriptives: by centre

<table>
<thead>
<tr>
<th>Variable</th>
<th>Manchester N=389</th>
<th>Mean (SD)</th>
<th>Leuven N=40</th>
<th>Mean (SD)</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testosterone (nmol/L)</td>
<td>17.3 (6.2)</td>
<td>18.6 (5.9)*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Free testosterone (pmol/L)</td>
<td>306.1 (91.1)</td>
<td>324.8 (88.6)*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bioavailable testosterone (nmol/L)</td>
<td>7.6 (2.3)</td>
<td>8.2 (2.3)*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oestradiol (pmol/L)</td>
<td>80.4 (25.7)</td>
<td>73.5 (24.2)*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Free oestradiol (pmol/L)</td>
<td>1.4 (0.4)</td>
<td>1.2 (0.4)*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bioavailable oestradiol (pmol/L)</td>
<td>56.4 (18.0)</td>
<td>51.2 (17.0)*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SHBG (nmol/L)</td>
<td>42.0 (18.2)</td>
<td>43.7 (19.2)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Reference range in healthy men aged 18–29 years for total testosterone measured by mass spectroscopy (MS) is 9–42 nmol/L and for calculated free testosterone 146–555 pmol/L [36]. There are at present no published reference ranges for oestradiol measured by MS and for calculated free testosterone 146–555 pmol/L [36]. There are at present no published reference ranges for SHBG measured by immunoassay is 13–53 nmol/L [37]. *p<0.05

Table 3  Influence of age on pQCT parameters at the radius: by centre

<table>
<thead>
<tr>
<th>Variable</th>
<th>Manchester</th>
<th></th>
<th></th>
<th>Leuven</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β co-efficient (95% CI)</td>
<td>% change/year</td>
<td>β co-efficient (95% CI)</td>
<td>% change/year</td>
<td></td>
</tr>
<tr>
<td><strong>Midshaft radius</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cortical BMD</td>
<td>−1.210 (−1.573, −0.846)*</td>
<td>−0.107</td>
<td>−0.894 (−1.225, −0.562)*</td>
<td>−0.077</td>
<td></td>
</tr>
<tr>
<td>Cortical BMC</td>
<td>−0.290 (−0.462, −0.119)*</td>
<td>−0.271</td>
<td>−0.260 (−0.414, −0.108)*</td>
<td>−0.208</td>
<td></td>
</tr>
<tr>
<td>Total area</td>
<td>0.176 (0.384, 0.384)</td>
<td>0.119</td>
<td>0.060 (0.142, 0.261)</td>
<td>0.040</td>
<td></td>
</tr>
<tr>
<td>Cortical thickness</td>
<td>−0.010 (−0.014, −0.005)*</td>
<td>−0.319</td>
<td>−0.007 (−0.010, −0.003)*</td>
<td>−0.219</td>
<td></td>
</tr>
<tr>
<td>Medullary area</td>
<td>0.310 (0.147, 0.473)*</td>
<td>0.824</td>
<td>0.206 (0.036, 0.375)*</td>
<td>0.471</td>
<td></td>
</tr>
<tr>
<td>Stress strain index</td>
<td>−0.022 (−0.637, 0.593)</td>
<td>−0.021</td>
<td>−0.510 (−1.114, 0.094)</td>
<td>−0.148</td>
<td></td>
</tr>
<tr>
<td>CSMA*</td>
<td>−20.561 (−26.464, −14.658)*</td>
<td>−0.567</td>
<td>−14.763 (−19.908, −9.618)*</td>
<td>−0.394</td>
<td></td>
</tr>
<tr>
<td><strong>Distal radius</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total density</td>
<td>−1.847 (−2.498, −1.196)</td>
<td>−0.446</td>
<td>−1.665 (−2.157, −1.172)*</td>
<td>−0.461</td>
<td></td>
</tr>
<tr>
<td>Total area</td>
<td>0.413 (0.094, 0.921)</td>
<td>0.114</td>
<td>0.501 (0.102, 1.103)</td>
<td>0.121</td>
<td></td>
</tr>
<tr>
<td>Trabecular density</td>
<td>−0.676 (−1.137, −0.216)*</td>
<td>−0.397</td>
<td>−0.452 (−0.825, −0.079)*</td>
<td>−0.220</td>
<td></td>
</tr>
</tbody>
</table>

*p<0.05

*Change in each pQCT parameter per 1 year increase in age

b Cross-sectional muscle area
Statistical analysis

Linear regression was used to explore the association between age and various pQCT parameters as dependent variables; and the results expressed as unstandardised $\beta$ coefficients and 95% confidence intervals. Regression analysis was also used to investigate the association between pQCT parameters and sex hormones (analysed as continuous variables) including total, free and bioavailable E$_2$ and T. Adjustments were made in these analyses for age,

Fig. 1  a Association between cortical BMD at the midshaft radius and age: by centre. b Association between stress strain index at the midshaft radius and age: by centre. c Association between cross-sectional muscle area at the midshaft radius and age: by centre. d Association between total density at the distal radius and age: by centre.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Manchester</th>
<th>Leuven</th>
<th>$\beta$ coeff</th>
<th>$p$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortical BMD (mg/cm$^3$)</td>
<td></td>
<td></td>
<td>-1.210</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Stress strain index (mm$^3$)</td>
<td></td>
<td></td>
<td>-0.022</td>
<td>NS</td>
</tr>
<tr>
<td>Cross-sectional muscle area (mm$^2$)</td>
<td></td>
<td></td>
<td>-20.566</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
height and weight as these variables were found to have significant independent associations with the pQCT parameters. We tested for a centre interaction for the hormone and pQCT regressions. For some parameters, there was a significant interaction and therefore our analyses were performed in each centre separately. Based on previous data suggesting an influence of age on the association between sex hormone status and pQCT parameters, the analysis was repeated after stratification by age (<60 and >60 years) [14]. Subjects were categorised into those above or below a bioE$_2$ threshold, defined as the median value in those over 60 years (51 pmol/L) and the association between bioE$_2$ and BMD measurements (at both 4% and 50% sites) examined. All data from the two centres were analysed separately. Statistical analysis was performed using STATA version 9.2 (http://www.stata.com).

Results

Subject characteristics

Three hundred thirty-nine men from Manchester and 389 from Leuven participated in this study. Their mean ages were 60.2 and 60.0 years, respectively. There were no differences in height or weight between subjects recruited in the two centres, but body mass index was slightly greater in Manchester (27.5 vs 26.9 kg/m$^2$), see Table 1. Cortical BMD and BMC at the midshaft, and also cross-sectional muscle area and SSI were significantly greater in subjects recruited in Leuven, Table 1. At the distal radial (4%) site, radial area was greater in Leuven and total BMD lower in Leuven compared to Manchester, indicating the slightly different scan location (in more distal thus expanded radius site in Leuven).

Sex hormone levels in the different centres are presented in Table 2. The mean serum T levels (total, free and bioavailable) were higher in Leuven than Manchester while the total, free and bioavailable E$_2$ levels were lower. There was no difference in SHBG levels in the two centres.

Age-related variations in bone mass and geometry

At the 50% midshaft site, lower cortical BMD, BMC, thickness and muscle area, and greater medullary area were decreased with age. There were no age-related variations in bone strength as assessed by SSI, (Table 3, Fig. 1) at either study centre. There were small though non-significant increases in bone area with age. For all parameters the change with age was broadly linear across the age range with no evidence of accelerated loss in later life. At the distal radius, there was a negative association of both trabecular and total BMD with age in both centres, Fig. 1.

Influence of sex hormones on pQCT parameters

The association between total, free, and bioavailable fractions of T and E$_2$ with pQCT parameters were broadly similar. We present data here for the bioavailable hormone relationships (bioE$_2$, bioavailable testosterone (bioT)) (Table 4). In Leuven men, higher bioE$_2$ was associated with increased cortical BMD at the 50% site and trabecular BMD at the 4% site; higher bioE$_2$ was associated also with greater cortical thickness and smaller medullary area. There was no important effect of bioT on BMD at either site. BioT was positively associated with CSMA in the Leuven men. There were no significant associations with any of the skeletal parameters in the Manchester men other than a negative association between total area (4% site) and bioE$_2$. Based on previous data [14] suggesting an influence of age on the association between sex hormone status and pQCT parameters, we analysed men above and below 60 years separately. The data are presented in Table 5. In Leuven men, all the significant
associations observed in the unstratified analysis were observed exclusively in the older men. Furthermore, among Leuven men older than 60 years, a number of significant associations emerged that were not present in the unstratified analysis. There was a positive association between bioE\(_2\) and cortical BMC at the 50% site and total BMD at the 4% site. There were positive associations also between bioT and (1) cortical BMC and stress strain index at the 50% site and (2) total area at the 4% site.

**Table 4 Influence of bioavailable testosterone and oestradiol on pQCT parameters at the radius: by centre**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Manchester (\beta) co-efficient (95% CI)</th>
<th>Leuven (\beta) co-efficient (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Midshaft radius</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cortical BMD</td>
<td>-0.427 (−2.505, 1.651)</td>
<td>0.583 (−1.354, 2.519)</td>
</tr>
<tr>
<td>BioT</td>
<td>-0.006 (−0.237, 0.225)</td>
<td>0.393 (0.167, 0.618)*</td>
</tr>
<tr>
<td>BioE(_2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cortical BMC</td>
<td>0.235 (−0.676, 1.145)</td>
<td>0.812 (−0.009, 1.633)</td>
</tr>
<tr>
<td>BioT</td>
<td>-0.056 (−0.157, 0.046)</td>
<td>0.094 (−0.002, 0.190)</td>
</tr>
<tr>
<td>BioE(_2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total area</td>
<td>0.140 (−0.934, 1.214)</td>
<td>0.511 (−0.590, 1.612)</td>
</tr>
<tr>
<td>BioT</td>
<td>-0.072 (−0.191, 0.047)</td>
<td>−0.107 (−0.236, 0.022)</td>
</tr>
<tr>
<td>BioE(_2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cortical thickness</td>
<td>-0.002 (−0.026, 0.023)</td>
<td>0.018 (−0.004, 0.040)</td>
</tr>
<tr>
<td>BioT</td>
<td>-0.001 (−0.004, 0.002)</td>
<td>0.004 (0.001, 0.006)*</td>
</tr>
<tr>
<td>BioE(_2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medullary area</td>
<td>0.028 (−0.840, 0.896)</td>
<td>−0.160 (−1.145, 0.825)</td>
</tr>
<tr>
<td>BioT</td>
<td>-0.030 (−0.127, 0.066)</td>
<td>−0.156 (−0.272, −0.040)*</td>
</tr>
<tr>
<td>BioE(_2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stress strain index</td>
<td>1.090 (−2.139, 4.319)</td>
<td>2.541 (−0.730, 5.812)</td>
</tr>
<tr>
<td>BioT</td>
<td>-0.184 (−0.543, 0.175)</td>
<td>−0.106 (−0.485, 0.274)</td>
</tr>
<tr>
<td>BioE(_2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CSMA(^b)</td>
<td>4.020 (−25.383, 33.424)</td>
<td>31.382 (7.565, 55.198)*</td>
</tr>
<tr>
<td>BioT</td>
<td>-2.073 (−5.334, 1.188)</td>
<td>1.099 (−1.733, 3.931)</td>
</tr>
<tr>
<td>BioE(_2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Distal radius</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total density</td>
<td>0.288 (−3.397, 3.974)</td>
<td>−0.472 (−3.261, 2.317)</td>
</tr>
<tr>
<td>BioT</td>
<td>0.248 (−0.161, 0.656)</td>
<td>0.259 (−0.069, 0.586)</td>
</tr>
<tr>
<td>BioE(_2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total area</td>
<td>-0.295 (−2.994, 2.403)</td>
<td>3.241 (−0.107, 6.590)</td>
</tr>
<tr>
<td>BioT</td>
<td>-0.313 (−0.611, −0.015)*</td>
<td>0.134 (−0.263, 0.531)</td>
</tr>
<tr>
<td>BioE(_2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trabecular density</td>
<td>0.590 (−2.043, 3.224)</td>
<td>0.399 (−1.742, 2.540)</td>
</tr>
<tr>
<td>BioT</td>
<td>0.087 (−0.206, 0.379)</td>
<td>0.316 (0.064, 0.568)*</td>
</tr>
<tr>
<td>BioE(_2)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(p<0.05\)

\(^a^\) Adjusted for age, height, and weight

\(^b^\) Cross-sectional muscle area

Influence of threshold level of bioavailable oestradiol

The median bioE\(_2\) in men (both centres combined) over 60 years was 51 pmol/L. Among the Leuven men, after adjusting for age, height, and weight there was a significant association between bioE\(_2\) and cortical BMD at the 50% site among those above and below this threshold. The magnitude of effect associated with trabecular BMD at the 4% site was broadly similar among those above and below the threshold, but because of smaller numbers neither association was statistically significant, see Table 6. There was no association between bioE\(_2\) and BMD in either compartment in Manchester men.

**Discussion**

Our data confirm evidence of age-related change at the midshaft radius in cortical BMD and BMC, cortical thickness and medullary area in middle-aged and elderly men. Among older Leuven men oestrogen appeared to play a role in maintaining BMD. BioT had no effect on BMD, but may influence bone health through an effect on muscle mass and bone area.

Our data confirm that there is lower BMC, thinner cortex and larger medullary area at the radial diaphysis, and also a lower muscle area in ageing men. Despite the lower BMC and muscle area with ageing it is possible bone strength is maintained through periosteal apposition (indicated by marginally greater bone area in older men). These data are consistent with those previously reported at the distal site, with loss of trabecular and total BMD [29] and a gain of bone on the periosteal surface. The periosteal apposition at this site has been shown to maintain strength and may be one reason why the incidence of forearm fracture is lower in men (than women) [30]. Taking muscle area as a surrogate for loading, it is plausible that the small adaptations in bone geometry can maintain strength to the level required for reduced loading from muscles.

Evidence from observational and clinical studies support the view that oestrogen is the most important sex steroid in determining bone mass in men [7–12]. Our finding of a positive relationship between E\(_2\) and BMD at both the 4% and 50% sites in the Leuven men is consistent with this view. The association with bioE\(_2\) was stronger in the older, than the younger, men which would be consistent with a lower level of bioavailable hormone in older age. Khosla et al. reported evidence of a threshold level of bioE\(_2\) (30 pM) above which no association with oestrogen was observed in cortical BMD, but not trabecular, bone at a range of skeletal sites (femoral neck, distal radius, and distal tibia) [14]. The threshold used, defined as the...
median value in men age 60 years, differed from the value used in our study (51 pmol/L) which was based on mass spectrometry measurements. Our data do not support these observations of a threshold effect of bioEF on cortical bone.

The current view is that testosterone acts on bone primarily via aromatisation to estrogens. There is some evidence, at least in rats, that T may increase periosteal apposition (and thereby increase total area), and certainly in adolescents T increases periosteal growth. Szulc et al. using data from DXA, suggested an increase in periosteal apposition with age though not via an action of T [15, 31]. In contrast, Khosla et al. found an inverse association in men with higher levels of T linked with reduced bone area [14]. Our results (both centres) showed no significant change in bone area with increasing testosterone at the 50% site though there was a positive association at the 4% site among the older Leuven men.

One of the intriguing findings was the differences in the absolute pQCT parameters between the two centres and the
relationships with sex steroids. Subjects in both centres were recruited using the same methods and were from a similar socioeconomic background. Removing subjects (n = 18) who were taking medications known to influence sex steroid levels did not change the results. Further adjustment for smoking and physical activity had no effect on these relationships. The lower total BMD and larger bone area in Leuven at the 4% site may in part be related to the slightly different and more distal slice location used at the two centres. It is unlikely, however, that this difference in protocol explains centre differences at the 50% site due to the more homogenous structure of the radius at this anatomical site. It is therefore likely that other explanations, including genetic and environmental factors, play a role in these Manchester–Leuven skeletal and hormone differences. Genetic factors are known to influence both bone mass and structure at the radius. Data from family and twin studies suggest that genetic factors explain about 50% of the variation in the radius total and trabecular vBMD, and up to 40% of cortical vBMD [32, 33]. In addition, a large proportion of the variation in geometric parameters such as radius cross-sectional area (27%) and cortical thickness (51%) are also attributable to genetic factors [33]. Variations in other skeletal parameters across Europe have previously been reported [34]; however, to the best of our knowledge, there are no data concerning pQCT parameters. We cannot explain the variation in findings in relation to the associations between bone parameters and sex hormones, other than the slight difference in protocol using pQCT which we feel would be unlikely to explain the variation. The similarity in rate of change with age for the skeletal parameters in both centres provides some construct validity to these measures.

The strength of our study was that it was population based and used pQCT measurements to obtain information not only on bone density but also bone morphology. There are some limitations which need to be considered when interpreting the results. The response rate for participation in the study was 45% [20]. Those who participated may have differed with respect to bone health and/or sex hormone status than those who did not participate. However, the main findings, in relation to the sex steroid levels were based on internal comparisons among respondents and so selection factors are unlikely to have had an important effect. One of the key factors in designing the study was to ensure standardisation of the study instruments used in the different participating centres. Hormone measurements were performed in a central reference laboratory to minimise assay variability. The same pQCT scanner type and model was used in each centre and after testing scanner differences with the EFP, no cross-calibration was necessary. There was a small difference in the 4% and 50% site location between centres, Leuven being 1–2 mm more distal in position than Manchester, as evidenced by a larger radial area and a lower total BMD in Leuven compared to Manchester. This emphasizes the need to have very precise and detailed protocols, including an image of the position of the reference line, for performing single-slice pQCT in multiple centres; quite large differences in the measured parameters can be observed in the 4% site, even in adjacent slices [35]. Although this may explain differences in BMD and area at the 4% site between centres, it is unlikely to affect the relationship between these parameters and sex hormones at the 50% site. Our study was cross-sectional: to determine true age-related changes in bone health prospective data are needed. The results were also obtained from a predominantly Caucasian European population so cannot be extrapolated beyond this setting.

In conclusion, there is evidence of age-related change at the midshaft radius in cortical BMD and BMC, cortical thickness and medullary area in middle-aged and elderly European men. Among older men, bioE2 may play a role in maintaining cortical and trabecular BMD. BioT has no effect on BMD but may influence bone health through an effect on muscle mass and bone area.
The European Male Ageing Study (EMAS) is funded by the Commission of the European Communities Fifth Framework Programme “Quality of Life and Management of Living Resources” Grant QLK6-CT-2001-00258 and supported by funding from Arthritis Research UK. For additional information regarding EMAS contact Frederick Wu, MD; Dept of Endocrinology, Manchester Royal Infirmary, UK. The authors wish to thank the men who participated in the eight countries, the research/nursing staff in the eight centres: C Pott, Manchester, E Wouters, Leuven, M Nilsson, Malmo, M del Mar Fernandez, Santiago de Compostela, M Jedrzejowska, Lodz, H-M Tabo, Tartu, A Heredi, Szeged for their data collection and C Moseley, Manchester for data entry and project coordination. The pQCT measurements were funded through a research grant from Central Manchester Universities Hospitals NHS Foundation Trust Endowment Funds, and the bone densitometry database was prepared for analysis by Mr. Mike Machin.

Dr. Vanderschueren is a senior clinical investigator supported by the Clinical Research Fund of the University Hospitals Leuven, Belgium. Dr. Boonen is a senior clinical investigator of the Fund for Scientific Research-Flanders, Belgium (F.W.O.-Vlaanderen). Dr. Boonen is holder of the Leuven University Chair in Metabolic Bone Diseases.

Conflicts of interest None.

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References


Publication 3: Influence of bone remodelling rate on quantitative ultrasound parameters at the calcaneus and DXA BMD of the hip and spine in middle-aged and elderly European men: the European Male Ageing Study (EMAS)
CLINICAL STUDY

Influence of bone remodelling rate on quantitative ultrasound parameters at the calcaneus and DXA BMDa of the hip and spine in middle-aged and elderly European men: the European Male Ageing Study (EMAS)

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†(See acknowledgements section for details of the EMAS group)

Abstract

Objective: To assess the influence of sex hormones on markers of bone turnover and to explore the association between these markers and bone health in middle-aged and elderly European men.

Design: A cross-sectional population-based survey.

Methods: Men aged 40–79 years were recruited from population registers in eight European centres. Subjects completed a postal questionnaire which included questions concerning lifestyle and were subjected to undertake quantitative ultrasound (QUS) of the calcaneus and to provide a fasting blood sample from which the bone markers serum N-terminal propeptide of type 1 procollagen (P1NP) and crosslinks (β-C-terminal cross-linked telopeptide (β-cTX)), total testosterone, total oestradiol (E2), sex hormone-binding globulin (SHBG) and insulin-like growth factor 1 (IGF1) were measured. Dual-energy X-ray absorptiometry (DXA) of the hip and lumbar spine was performed in two centres.

Results: A total of 3120, mean age 59.9 years (S.D. = 11.0) were included. After adjustment for centre, age, height, weight, lifestyle factors, season and other hormones, total and free E2 were negatively associated with β-cTX but not P1NP while SHBG, IGF1 and parathyroid hormone (PTH) were positively associated with both β-cTX and P1NP. Total or free testosterone was not independently associated with either bone marker. After the same adjustments, higher levels of both bone markers were significantly associated with lower QUS parameters and lower DXA-assessed bone density at the total hip and lumbar spine.

Conclusions: E2, SHBG, IGF1 and PTH contribute significantly to the regulation/rate of bone turnover in middle-aged and older European men. Higher rates of bone remodelling are negatively associated with male bone health.

European Journal of Endocrinology 165 977–986
Introduction

In recent years, a number of cross-sectional studies (1–6) have assessed bone turnover markers in men, focusing on age-related variations, potential determinants of bone remodelling and the association between bone turnover and areal bone mineral density (BMDa). These studies have shown serum levels of bone turnover markers to be high in young adult men followed by a prompt decline, at least until the age of 40 (1–6). From 40 years on, age-related patterns in men become less consistent. Average concentrations of bone turnover markers in older men have been shown to be either relatively stable, or to slightly decrease or increase (7, 8). These various age-related trends are probably due to the cumulative effects of several factors: real changes in bone remodelling, changes in the activity of enzymes involved in the metabolism of bone collagen, age-related decline in glomerular filtration and, for urinary markers, age-associated reduction in muscle mass leading to lower urinary creatinine excretion (9).

While age-related trends have been somewhat discordant, studies have consistently shown a large scatter of individual values of bone turnover marker levels. The biological significance of this scatter, however, remains unclear. One possibility is that it reflects the multiplicity of determinants of bone turnover in men. Hormonal factors, such as reduced exposure to free 17β-oestradiol (E2) and free testosterone and lifestyle factors are amongst the candidates that may affect bone remodelling rate (10–12). Most analyses, however, have focused on just one or two selected potential determinants of bone turnover, with scant data on the cumulative interdependent effects of various factors and virtually no data on a number of specific potential confounders, such as serum insulin-like growth factor 1 (IGF1) (13).

Most reports assessing skeletal integrity according to levels of bone turnover have used dual-energy X-ray absorptiometry (DXA) measures of BMDa (1, 3, 14–19). In older men, increased bone turnover markers have been shown to be associated with lower BMDa and, more recently, poor bone microarchitecture (1, 3, 14–16). In line with these cross-sectional findings, prospective data have confirmed that higher levels of bone remodelling may be associated with increased rates of bone loss, although evidence for an increased risk of fracture is lacking (17–19). Data on the relationship between bone turnover rate and bone quantitative bone ultrasound (QUS) measurements are more limited, especially in men. In older women, bone turnover has been shown to be negatively related with calcaneal QUS in some but not all studies (20–25). In one study in men, QUS measurements were negatively associated with alkaline phosphatase, but the small sample size did not allow adjustment for potential confounding factors (26).

The objectives of this study were i) to characterise the distribution of bone turnover markers by age in middle-aged and elderly men, ii) to assess determinants of the bone turnover rate and iii) to explore the cross-sectional associations between bone turnover rate and male skeletal integrity as assessed by QUS and DXA in the context of the European Male Ageing Study (EMAS), a large population-based multinational cohort of men aged 40–79 years.

Materials and methods

Subjects

The subjects included in this analysis were recruited for participation in EMAS. Details concerning the study design and recruitment have been described previously (27). Briefly, men were recruited from population-based sampling frames in eight centres: Florence (Italy), Leuven (Belgium), Lodz (Poland), Malmö (Sweden), Manchester (UK), Santiago de Compostela (Spain), Szeged (Hungary) and Tartu (Estonia). Participating centres were selected to provide geographical and socioeconomic diversity within Europe, and their facilities to perform epidemiological surveys. Stratified random sampling was used with the aim of recruiting equal numbers of men in each of four 10-year age bands: 40–49, 50–59, 60–69 and 70–79 years. Subjects were invited by letter to complete a postal questionnaire and attend for an interviewer-assisted questionnaire. Ethics approval for the study was obtained in accordance with local institutional requirements in each centre. All subjects provided written informed consent.

Study questionnaires and clinical data

The postal questionnaire included questions concerning current smoking and alcohol consumption in the previous year (response set, every day/5–6 days per week/3–4 days per week/1–2 days per week/less than once a week/not at all). The subjects were also asked if they were currently being treated for a range of medical conditions that included diabetes and prostate disease. Information about medications was also collected. Height and weight were measured in a standardised fashion. Height to the nearest 1 mm using a stadiometer (Leicester height measure, SECA, Birmingham, UK Ltd) and body weight to the nearest 0.1 kg using an electronic scale (SECA, model no. 8801321009, SECA).

Hormone measurements

A single fasting morning (before 10:00) venous blood sample was obtained from all subjects. Serum was separated immediately after phlebotomy and stored at −80 °C until assay at the end of the baseline study.
Measurement of testosterone and E₂ were carried out by gas chromatography-mass spectrometry as described by Labrie et al. (28, 29). The lower limit of testosterone quantitation was 0.17 nmol/l and E₂ was 7.34 pmol/l. The coefficients of variation (CV) of testosterone measurements were 2.9% within runs and 3.4% between runs, and for E₂, were 3.5% within runs and 3.7% between runs. Sex hormone-binding globulin (SHBG) was measured by the Modular E170 platform electrochemiluminescence immunoassay (Roche Diagnostics) as described previously (30). Within- and between-assay CV for SHBG measurements were 1.70 and 3.18% respectively. The free and bioavailable (non-SHBG-bound) testosterone and E₂ levels were derived from total hormone, SHBG and albumin concentrations using mass action equations and association constants as described by Vermeulen et al. (31) and Van Pottelbergh et al. (32). In addition, samples were transported in frozen state to a single laboratory for measurement of IGF1 and parathyroid hormone (PTH; University of Santiago de Compostela). Serum was assayed for IGF1 using chemiluminescence. Within- and between-assay CV for IGF1 were 7.4 and 2.9% respectively. The detection limit of the assay was 20 ng/ml. Serum was assayed for PTH using a chemiluminescence immunoassay (Nichols Advantage Bio-Intact PTH assay, Quest Diagnostics, Madison, NJ, USA). Intra- and inter-assay CV for PTH were 6 and 2.8% respectively. The detection limit of the assay was 5 ng/ml.

**Bone marker measurements**

To assess bone resorption, serum β C-terminal cross-linked telopeptide (β-CTX) was measured on the Elecsys 2010 automated analyser (Roche Diagnostics GmbH) using the β-Crosslapses/serum reagents (33). This assay is specific for cross-linked β-isomerised type I collagen C-telopeptide fragments and uses two MAbs, each recognising the Glu-Lys-Ala-His-βAsp-Gly-Gly-Arg peptide (Crosslaps antigen). The intra-assay CV evaluated by repeated measurements of several serum samples was < 5.0%. The detection limit was 10 pg/ml. To evaluate bone formation, measurements were performed on the Elecsys 2010 with a two-site assay using MAbs raised against intact human N-terminal propeptide of type I procollagen (P1NP) purified from human amniotic fluid. This assay detects both intact monomeric and trimeric forms (total P1NP), as described previously (34). The inter-assay CV was < 3.0% and the lower detection limit < 5 ng/ml.

**Quantitative ultrasound of the heel**

QUS of the left heel was performed with the Sahara Clinical Sonometer (Hologic, Inc., Waltham, MA, USA) using a standardised protocol in all centres. Each centre calibrated the device daily with the physical phantom provided by the manufacturer and the performances of the devices was found to be stable. Outputs included the rate of loss of ultrasonic intensity with frequency (broadband ultrasound attenuation (BUA) measured in decibels per megahertz using Fourier transformation of the recorded signal) and the velocity of ultrasound transmission through bone (speed of sound (SOS) measured in meters per second from the sound propagation time between the transducers). In addition, estimated heel bone mineral density (eBMDₐ) in grams per square centimetre, was derived from the BUA and SOS measurements: eBMDₐ = 0.002592 × (BUA + SOS)– 3.687. Short-term precision of the method was established by duplicate measurements performed in 20 randomly selected cohort members in Leuven. The in vivo CV were 2.8 and 0.3% for BUA and SOS, respectively, and 3.4% for eBMDₐ. Repeat measurements were performed on a roving phantom at each of the eight centres (35). Standardised CV (SCV) for within machine variability ranged by centre: for SOS, from 1.0 to 5.6%, and BUA from 0.7 to 2.7%. SCV for between machine variability were 4.8% for BUA and 9.7% for SOS (35).

**Dual-energy X-ray absorptiometry**

BMDₐ scans were carried out in the Manchester and Leuven subsets of EMAS (n = 676). Both sites used DXA QDR 4500A devices from the same manufacturer (Hologic, Inc.). BMDₐ was measured at the lumbar spine (L1–L4) and proximal femur (total region). All scans and analysis were performed by trained and certified DXA technicians. The Hologic Spine Phantom was scanned daily to monitor the device performance and long-term stability. With our equipment, the precision errors of these measurements in Leuven were 0.57 and 0.56% at the lumbar spine and total femur region respectively. In Manchester, these precision errors were 0.97 and 0.97% respectively. Both devices were cross-calibrated with the European Spine Phantom (36).

**Statistical analysis**

Descriptive statistics were used to summarise subject characteristics including the distribution of bone turnover markers (P1NP and β-CTX), heel QUS parameters (BUA, SOS and eBMDₐ), DXA BMDₐ at the total hip and lumbar spine, sex hormone levels and IGF1. The association between age and bone marker levels, and also sex hormones and bone markers was assessed visually using scatter plots, superimposing linear lines and also locally-weighted scatter plot smooth (LOWESS) curves to examine potential nonlinearity. The strength of the associations was assessed using linear regression (with the bone turnover marker as dependent variable) and results expressed as β coefficients. In subsequent analyses for ease of interpretation and comparison we
standardised hormone measures and bone turnover markers into Z scores. To examine potential nonlinear/threshold effects we categorised these variables into tertiles and quintiles. Multivariable linear regression was then used to determine the association between hormone levels (separate models for each of the sex hormones, SHBG and IGF1) and bone turnover markers, adjusting for potential confounders including age, height, weight, centre, season of measurement and lifestyle characteristics – with the bone turnover marker as dependent variable. We then used a model that included all measured hormones to determine their independent associations with bone marker levels. Multivariable linear regression was then used to determine the association between bone turnover markers and QUS/DXA parameters with adjustments initially for centre, age, height, weight, lifestyle factors and season of measurement and then with further adjustments for the sex hormones and IGF1, to determine whether or not the associations between bone turnover markers and bone health were influenced by these factors. For all linear regression models, the distribution of the residuals was assessed by plotting quantiles of the standardised residuals against quantiles of a normal distribution, visually assessing if the plot deviated from a straight line and then statistically testing for deviation from normality by the Shapiro–Wilk test. There was no important deviation from the normality assumption in any of the reported results. For all multivariable models, the variance inflation factor was calculated to quantify the severity of any potential multicollinearity and only models where the multicollinearity was low were included. Results of all linear regression analyses are expressed as β coefficients or standardised β coefficients and 95% confidence intervals (CIs). Statistical analysis was performed by STATA version 9.2 (http://www.stata.com).

Results

Subjects
A total of 3120 men with a mean age of 59.9 years (s.d. = 11.0) had complete bone marker and QUS data. Characteristics of the subjects are shown in Table 1. In addition, 21% reported that they currently smoke, while 56% of the men reported consuming alcohol at least 1 day/week, 8% reported currently being treated for diabetes, 12% for prostate disease and 4% reported currently taking corticosteroids.

Association between age, bone turnover and sex hormones
Neither P1NP nor β-cTX was linearly associated with age (Fig. 1). When mean levels were compared between subjects stratified into 5-year age bands a slight U-shaped pattern emerged (data not shown). Compared with subjects aged 60–65 (mean = 39.0 ng/ml; 95% CI = 37.4–40.7), those aged 40–45 had higher P1NP levels (mean = 45.5 ng/ml; 95% CI = 43.5–47.5; P < 0.001). Similarly, compared with those aged 60–65, those aged 75–79 had higher P1NP levels (mean = 42.8 ng/ml; 95% CI = 40.8–44.8; P < 0.01). The same pattern was observed for β-cTX. However, the magnitude of these associations was small and age accounted for <1% of the variation in bone turnover markers. As expected, levels of free testosterone decreased with age while total E2 increased with age (Fig. 1). Age was positively associated with SHBG and PTH levels and negatively associated with IGF1 (Fig. 1).

Association between sex hormones and bone turnover
In bivariate analyses, higher levels of total E2 were not significantly associated with β-cTX (β coefficient (β) = −0.201; 95% CI = −0.458, 0.056; P = 0.13); however, free E2 was associated with lower β-cTX (Fig. 2). Higher levels of SHBG were associated with higher β-cTX (Fig. 2). Total/free E2 was not associated with P1NP; but higher SHBG was significantly associated with higher P1NP (data not shown). Free testosterone was not associated with either β-cTX or P1NP (data not shown). In a multivariable model including age, centre, height, weight, current smoking, alcohol consumption and season, higher levels of both total and free E2 were associated with lower β-cTX, and higher levels of SHBG remained associated with higher

---

**Table 1 Subject characteristics. Data are presented as mean (S.D.)**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>3120</td>
</tr>
<tr>
<td>Age at interview (years)</td>
<td>59.9 (11.0)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>173.6 (7.3)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>83.2 (13.6)</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>27.6 (4.0)</td>
</tr>
<tr>
<td>Testosterone (nmol/l)</td>
<td>16.4 (6.0)</td>
</tr>
<tr>
<td>Free testosterone (pmol/l)</td>
<td>289.9 (88.5)</td>
</tr>
<tr>
<td>Bioavailable testosterone (nmol/l)</td>
<td>7.1 (2.2)</td>
</tr>
<tr>
<td>Oestradiol (E2; pmol/l)</td>
<td>73.3 (24.5)</td>
</tr>
<tr>
<td>Free E2 (pmol/l)</td>
<td>1.3 (0.4)</td>
</tr>
<tr>
<td>Bioavailable E2 (pmol/l)</td>
<td>50.7 (17.0)</td>
</tr>
<tr>
<td>SHBG (nmol/l)</td>
<td>42.8 (19.6)</td>
</tr>
<tr>
<td>Parathyroid hormone (pg/ml)</td>
<td>28.4 (11.9)</td>
</tr>
<tr>
<td>IGF1 (ng/ml)</td>
<td>133.0 (42.9)</td>
</tr>
<tr>
<td>QUS Estimated bone mineral density (g/cm²)</td>
<td>0.541 (0.135)</td>
</tr>
<tr>
<td>Broadband ultrasound attenuation (dB/MHz)</td>
<td>80.1 (18.9)</td>
</tr>
<tr>
<td>Speed of sound (m/s)</td>
<td>1550.5 (34.0)</td>
</tr>
<tr>
<td>DXA Total hip (g/cm²)</td>
<td>1.013 (0.145)</td>
</tr>
<tr>
<td>Lumbar spine (g/cm²)</td>
<td>1.053 (0.174)</td>
</tr>
<tr>
<td>Bone markers P1NP (ng/ml)</td>
<td>41.7 (17.6)</td>
</tr>
<tr>
<td>β-cTX (pg/ml)</td>
<td>352.1 (179.9)</td>
</tr>
</tbody>
</table>
Bone remodelling and ultrasound parameters: influence of sex hormones, IGF1 and PTH

Association between bone turnover, ultrasound parameters and DXA-assessed bone density: influence of sex hormones, IGF1 and PTH

After the multivariable model had been further adjusted for free testosterone, free E2, SHBG, IGF1 and PTH, higher levels of β-cTX remained associated with lower BUA, SOS and eBMDa (Table 3). Similarly, higher levels of P1NP remained associated with lower SOS and eBMDa, however, the association with BUA became non-significant. In similar models, higher levels of both bone turnover markers remained associated with significantly lower DXA-measured BMDa at the total hip and lumbar spine. Overall, age, lifestyle, hormones and bone markers accounted for 11–25% of the variability in QUS/DXA parameters. Excluding subjects taking corticosteroids or reporting being treated for diabetes or prostate disease did not influence the results.

Discussion

In the large population-based EMAS cohort of ageing men, age, lifestyle and key hormones regulating bone metabolism jointly accounted for between 8 and 20% of the variability in bone turnover rate. E2, SHBG, IGF1 and PTH (but not testosterone, total or free) were identified as independent predictors of bone remodelling. In multi-adjusted models, which included hormone levels as covariates, higher levels of bone remodelling were significantly and negatively associated with QUS parameters and DXA-assessed BMDa.

Overall, bone turnover marker levels were relatively stable with age in the investigated age range. Several studies have provided evidence that levels of bone turnover markers were negatively associated with the QUS parameters (Table 3). Higher levels of P1NP were associated with lower BUA, SOS and eBMDa. Likewise, higher levels of β-cTX were associated with lower BUA, SOS and eBMDa. Similar results were observed with DXA BMDa at both the total hip and lumbar spine sites (Table 3). There was no evidence of any threshold effect when the bone turnover markers were included in the models categorised into either tertiles or quintiles.
Table 2  Association between hormones and bone turnover markers. Results expressed as $\beta$ coefficients (95% CI). Statistically significant values are presented in boldface.

<table>
<thead>
<tr>
<th>Independent variables (per S.D.)</th>
<th>Adjusted for age, centre, height, weight, current smoking, alcohol consumption and season</th>
<th>Further adjusted for FT, free E$_2$, SHBG, IGF1 and PTH</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1NP (per s.d.)</td>
<td>$\beta$-cTX (per s.d.)</td>
<td>P1NP (per s.d.)</td>
</tr>
<tr>
<td>FT</td>
<td>$-0.036 (-0.077, 0.004)$</td>
<td>$-0.073 (-0.111, -0.034)^*$</td>
</tr>
<tr>
<td>Total E$_2$</td>
<td>$0.017 (-0.019, 0.053)$</td>
<td>$-0.060 (-0.094, -0.026)^*$</td>
</tr>
<tr>
<td>Free E$_2$</td>
<td>$-0.024 (-0.060, 0.013)$</td>
<td>$-0.083 (-0.118, -0.049)^*$</td>
</tr>
<tr>
<td>SHBG</td>
<td>$0.135 (0.095, 0.175)^*$</td>
<td>$0.083 (0.045, 0.121)^*$</td>
</tr>
<tr>
<td>IGF1</td>
<td>$0.070 (0.032, 0.107)^*$</td>
<td>$0.088 (0.052, 0.123)^*$</td>
</tr>
<tr>
<td>PTH</td>
<td>$0.114 (0.078, 0.150)^*$</td>
<td>$0.178 (0.144, 0.211)^*$</td>
</tr>
</tbody>
</table>

$*P<0.05$. FT, free testosterone.

Turnover are high in young adult men – higher in fact than in women of similar age which most likely reflects active bone remodelling during consolidation after growth arrest, which occurs with some delay in men compared with women – and then gradually decline (9, 37). This decrease is mostly observed before the age of 40 (1–4) and could not be assessed in our cohort of men who were aged between 40 and 79 years. When we compared mean levels of bone turnover between subjects stratified into 5-year age bands, a slight U-shaped pattern emerged, with levels declining until age 60–65 years and then slightly rising. A more marked resurgence of bone turnover has been previously observed in older men for some, but not all, bone turnover markers – mainly free and total deoxypyridinoline, and in some, but not all, cohorts – mainly in men over the age of 80 (1–4). Such a significant increase has been primarily observed in elderly men with impaired mobility and hormonal or nutritional insufficiencies (38–40), and may therefore not apply to the present cohort of relatively healthy community-dwelling men.

Most of the variability in bone turnover rate could not be accounted for, even when combining age, lifestyle and key hormones of bone metabolism data. This suggests a major role for other determinants such as genetic background, nutritional status, underlying (occult) comorbidities as well as normal biological short- and long-term variability in bone turnover rate (41). In line with the inhibitory effect of E$_2$ on bone resorption (42, 43), low free E$_2$ was negatively associated with levels of $\beta$-cTX (3, 44, 45). The association between P1NP and free E$_2$, however, was not significant, possibly reflecting the divergent effects of an oestrogen-induced reduction in the overall rate of bone remodelling, on the one hand, and an oestrogen-mediated increase in bone formation locally at the level of the individual bone remodelling units, on the other hand (42).

Free testosterone was not significantly related to any of the bone turnover markers, suggesting that age-associated changes in androgen status is unlikely to drive bone turnover independently in men. However, it should be noted that, in our cohort, most participants had testosterone levels within the normal range. Higher levels of some bone markers (mainly indices of bone resorption) have been shown in hypogonadal men (38, 46–48).

We observed a highly significant positive correlation between SHBG and bone turnover, in line with previous studies in men and consistent with evidence that SHBG...

Table 3  Association between bone markers, DXA and QUS parameters. Results expressed as $\beta$ coefficients (95% CI). Statistically significant values are presented in boldface.

<table>
<thead>
<tr>
<th>Independent variables</th>
<th>Adjusted for age, centre, height, weight, current smoking, alcohol consumption and season</th>
<th>Further adjusted for free testosterone, free E$_2$, SHBG, IGF1 and PTH</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1NP (per s.d.)</td>
<td>$\beta$-cTX (per s.d.)</td>
<td>P1NP (per s.d.)</td>
</tr>
<tr>
<td>QUS (n=3120)</td>
<td>$-0.040 (-0.073, -0.006)^*$</td>
<td>$-0.073 (-0.109, -0.037)^*$</td>
</tr>
<tr>
<td>BUA (per s.d.)</td>
<td>$-0.066 (-0.100, -0.032)^*$</td>
<td>$-0.098 (-0.134, -0.062)^*$</td>
</tr>
<tr>
<td>SOS (per s.d.)</td>
<td>$-0.055 (-0.089, -0.021)^*$</td>
<td>$-0.089 (-0.125, -0.053)^*$</td>
</tr>
<tr>
<td>eBMD$_a$ (per s.d.)</td>
<td>$-0.040 (-0.073, -0.006)^*$</td>
<td>$-0.073 (-0.109, -0.037)^*$</td>
</tr>
<tr>
<td>Total hip (per s.d.)</td>
<td>$-0.104 (-0.171, -0.037)^*$</td>
<td>$-0.135 (-0.212, -0.057)^*$</td>
</tr>
<tr>
<td>Lumbar spine (per s.d.)</td>
<td>$-0.086 (-0.158, -0.015)^*$</td>
<td>$-0.171 (-0.253, -0.089)^*$</td>
</tr>
</tbody>
</table>

$*P<0.05$.
is negatively related to bone density (49–51) and positively related to fracture risk (52). We recently reported a similar negative association with bone QUS parameters, even after adjusting for potential confounders, in our cohort (53). Similar findings have also been documented in ageing women (54). However, the mechanism of this association remains unclear. Part of the association is likely to reflect the fact that SHBG is the principal determinant of bioavailability of free sex steroids. However, even after adjusting for free E2 and free testosterone, SHBG remained strongly associated with bone turnover, suggesting that SHBG may potentially have a direct negative effect on bone, independent of sex steroids. Alternatively, calculated concentrations of circulating free sex steroid may not accurately reflect local bioavailability of these hormones at the target tissue level (55–57). Calculation of free sex steroid concentrations assumes that the binding affinity between both sex steroids and SHBG is constant in the entire population and does not vary with age while, in reality, it may be affected by genetic variants or isoforms of SHBG, age and levels of other SHBG-binding steroids (54, 58–61).

Previously, PTH contributed significantly to the bone turnover rate in some (62) but not all studies (2). Importantly, in this cohort, PTH was a significant determinant of bone turnover in multivariable models independently of other lifestyle and hormonal factors. This confirms that age-related secondary hyperparathyroidism is a significant determinant of age-related BMD decrease in men (62, 63). In line with the fact that IGF1 is known to act directly on bone cells and to stimulate bone remodelling, bone turnover marker levels were also found to be positively associated with IGF1 levels in this study. Although similar observations have been made in adolescents (64, 65), data in a general male population are very limited (2, 8).

In our population-based sample, both QUS parameters and DXA-assessed BMDa were inversely related to bone turnover rate, independent of age, weight, height, lifestyle factors and key hormones regulating bone turnover. These findings are concordant with previous studies (1, 3, 14–16) and confirm the importance of bone remodelling for bone health in men, although it should be noted that the proportion of the variance in bone parameters explained by our multivariable models was <26%. They show that the rate of bone turnover is a significant determinant of bone density, even in weight-bearing sites where bone metabolism is under a strong influence of the mechanical effect of body weight. While the associations between bone turnover and QUS tended to be slightly weaker than with DXA, findings were consistent across different skeletal sites and across different assessment methods, supporting the concept that QUS measurements in calcaneal bone should be primarily regarded as an indicator of bone density (66). In line with this concept, QUS parameters and DXA-assessed BMDa have been shown to be equally predictive for incident fractures, in both sexes (67–69).

The main strength of the current analysis is that it used well-established methods to assess the impact of a wide variety of potential determinants of bone remodelling and skeletal integrity in a large, community-based population of middle-aged and elderly men. Our finding that, even after adjustment for lifestyle and hormonal variables known to regulate male bone metabolism, bone markers remained significantly associated with bone health provide strong evidence that other factors influence bone remodelling and determine skeletal integrity. What constitutes this residual variance in bone turnover rate will require more research. Future studies should clarify the extent to which other determinants (e.g. genetic background, nutritional status and underlying comorbidities) contribute to skeletal maintenance in ageing men.

Our study also has limitations. Our findings are based on data in middle-aged and older European men and may not apply to other groups of men. Also, the participants were home-dwelling volunteers recruited in selected centres and may not be representative of the general population. A small number of men (n = 249; 7%) were excluded from the analysis due to incomplete data and it is possible that these men were different in terms of health status from those that were included. However, there was no difference in age, smoking status, alcohol consumption or hormone levels between those included and those excluded providing some reassurance against this. Bone density and turnover were assessed by established methods; however, bone mass was estimated only at weight-bearing sites and bone turnover analysed only by two biochemical markers. Bone density as assessed by DXA was performed in only a subset of the subjects. The free fractions of testosterone and E2 were calculated rather than measured and may not reflect the absolute values. Whilst a wide variety of potential determinants of bone health were assessed in EMAS, it is possible that unmeasured factors explain the observed results. Analysis of the influence of bone remodelling on osteoporotic fracture was not possible due to lack of prospective fracture data at this time. Finally, in a cross-sectional study, associations can be demonstrated but it is not possible to determine cause-and-effect relationships or to disentangle the temporal nature of the observed associations.

In conclusion, E2, SHBG, IGF1 and PTH contribute significantly and independently to bone turnover in middle-aged and older European men. Higher rates of bone remodelling are negatively associated with male bone health independently of age, lifestyle factors and hormonal exposure.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.
Funding
The European Male Ageing Study (EMAS) is funded by the Commission of the European Communities Fifth Framework Programme ‘Quality of Life and Management of Living Resources’ grant QLK5-CT-2001-00258. Additional support was also provided by Arthritis Research UK.

Acknowledgements
EMAS group: The Principal Investigator of EMAS is Prof. F C W Wu, MD; Department of Endocrinology, Manchester Royal Infirmary, Manchester, UK. The EMAS Group: Florence (Gianni Forti, Luisa Petrone, Giovanni Corona); Leuven (Dirk Vanderschueren, Steven Boonen, Herman Borghs); Lodz (Krzysztof Kula, Jolanta Slowikowska-Hilcaer, Renata Walczak-Jedrezewska); London (Ilpo Ruhtanen); Malmo (Aleksander Giwercman); Manchester (Frederick Wu, Alan Silman, Terence O’Neill, Joseph Finn, Philip Steer, Abdelouahid Tajar, David Lee, Stephen Pye); Santiago (Felipe Casanueva, Mary Lage, Ana I Castro); Szeged (Gyorgy Bartfai, Imre Foldesi, Imre Fejes); Tartu (Alpvilla-Negrin J, Gonza´lez-Reimers E, Santolaria-Ferna ´ndez F, Marchand F, Duboeuf F & Delmas PD).

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Received 20 April 2011
Revised version received 4 July 2011
Accepted 8 September 2011
Publication 4: Active vitamin D (1,25-dihydroxyvitamin D) and bone health in middle-aged and elderly men: the European Male Aging Study (EMAS)
Active Vitamin D (1,25-Dihydroxyvitamin D) and Bone Health in Middle-Aged and Elderly Men: The European Male Aging Study (EMAS)


Context: There is little information on the potential impact of serum 1,25-dihydroxyvitamin D [1,25(OH)₂D] on bone health including turnover.

Objective: The objective of the study was to determine the influence of 1,25(OH)₂D and 25-hydroxyvitamin D [25(OH)D] on bone health in middle-aged and older European men.

Design, Setting, and Participants: Men aged 40–79 years were recruited from population registers in 8 European centers. Subjects completed questionnaires that included questions concerning lifestyle and were invited to attend for quantitative ultrasound (QUS) of the heel, assessment of height and weight, and a fasting blood sample from which 1,25(OH)₂D, 25(OH)D, and PTH were measured. 1,25(OH)₂D was measured using liquid chromatography tandem mass spectrometry. Bone markers serum N-terminal propeptide of type 1 procollagen (P1NP) and crosslinks (β-cTX) were also measured. Dual-energy x-ray absorptiometry (DXA) of the hip and lumbar spine was performed in 2 centers.

Main Outcome Measure(s): QUS of the heel, bone markers P1NP and β-cTX, and DXA of the hip and lumbar spine were measured.

Results: A total of 2783 men, mean age 60.0 years (SD 11.0) were included in the analysis. After adjustment for age and center, 1,25(OH)₂D was positively associated with 25(OH)D but not with PTH. 25(OH)D was negatively associated with PTH. After adjustment for age, center, height, weight, lifestyle factors, and season, 1,25(OH)₂D was associated negatively with QUS and DXA parameters and associated positively with β-cTX. 1,25(OH)₂D was not correlated with P1NP. 25(OH)D was positively associated with the QUS and DXA parameters but not related to either bone turnover marker. Subjects with both high 1,25(OH)₂D (upper tertile) and low 25(OH)D (lower tertile) had the lowest QUS and DXA parameters and the highest β-cTX levels.

Conclusions: Serum 1,25(OH)₂D is associated with higher bone turnover and poorer bone health despite being positively related to 25(OH)D. A combination of high 1,25(OH)₂D and low 25(OH)D is associated with the poorest bone health. (J Clin Endocrinol Metab 98: 995–1005, 2013)

* D.V. and S.R.P. contributed equally to this manuscript.
† Author affiliations are shown at the bottom of the next page.

Abbreviations: BMDa, areal bone mineral density; BMI, body mass index; BUA, broadband ultrasound attenuation; CI, confidence interval; β-cTX, β-C-terminal cross-linked telopeptide; CV, coefficient of variation; DXA, dual-energy x-ray absorptiometry; E₂, estradiol; EMAS, European Male Aging Study; LC-MS/MS, liquid chromatography-tandem MS; MS, mass spectrometry; 25(OH)D, 25-hydroxyvitamin D; 1,25(OH)₂D, 1,25-dihydroxyvitamin D; PASE, Physical Activity Scale for the Elderly; P1NP, N-terminal propeptide of type 1 procollagen; QUS, quantitative ultrasound; SOS, speed of sound; T, testosterone.
Vitamin D deficiency is common, particularly among the elderly (1). Vitamin D status is most commonly characterized by measuring serum 25-hydroxyvitamin D [25(OH)D], the most abundant circulating metabolite. The influence of 25(OH)D on bone health has been extensively examined (1), particularly in postmenopausal women, with fewer studies in men (2–11).

1,25-Dihydroxyvitamin D [1,25(OH)₂D] is the metabolically active molecule responsible for most of the actions of vitamin D and is derived from 25(OH)D by 1α-hydroxylation primarily in the kidney (12). The main effect of 1,25(OH)₂D is to increase calcium absorption from the gut (13). 1,25(OH)₂D binds to the vitamin D receptor in the epithelial cells of the duodenum causing the synthesis of calcium binding proteins that regulate active intestinal calcium absorption (13, 14). It also stimulates calcium reabsorption in the kidney. The production of 1,25(OH)₂D is stimulated by PTH and its concentrations directly influenced by serum calcium and phosphate (15). In addition to regulating serum calcium uptake in the intestine and kidney, evidence from in vitro and animal studies suggest that 1,25(OH)₂D may also regulate calcium resorption from bone by having direct effects on bone cells (13, 14, 16). There is, however, little information on the potential impact of serum 1,25(OH)₂D on bone health including turnover.

Compared with 25(OH)D, serum concentrations of 1,25(OH)₂D are 1000-fold lower and its half-life much shorter at approximately 7 hours (12). Measurement of 1,25(OH)₂D has typically been by RIA, often preceded by HPLC, thus making it time consuming. Such measurement also typically required large volumes of serum. Recent advances in mass spectrometry (MS) have provided more accurate measurements of many metabolic hormones, but to date very few MS-based assays for 1,25(OH)₂D have been developed. Consequently, epidemiological data are scarce, but there is some evidence that 1,25(OH)₂D declines with age in some (17–19) but not all studies (20, 21). Among the few studies that have measured both vitamin D metabolites and also PTH, some provide evidence of a positive association between 1,25(OH)₂D and 25(OH)D (17–19) and between 1,25(OH)₂D and PTH (17, 18). There are very few studies examining the influence of 1,25(OH)₂D on bone health, and the data are conflicting. A small study of healthy men aged 30–92 years found no association between 1,25(OH)₂D and radial or vertebral bone mineral content (21). One study showed a doubling of the risk of hip fracture in postmenopausal women with low serum 1,25(OH)₂D (22), and another study found lower 1,25(OH)₂D levels in hip fracture patients compared with controls (23).

The European Male Aging Study (EMAS) is a large population-based study of aging in middle-aged and older European men, which incorporates an extensive range of clinical, biochemical, health, and lifestyle information, including a new state-of-the-art MS-based measurement of serum 1,25(OH)₂D. We used data from EMAS to examine the interrelationships between 1,25(OH)₂D, 25(OH)D, and PTH. We compared the influence of 1,25(OH)₂D, 25(OH)D, and PTH on bone health measured using quantitative ultrasound (QUS) of the heel, dual-energy x-ray absorptiometry (DXA) of the hip and lumbar spine, and serum markers of bone turnover.

Materials and Methods

Subjects

The subjects included in this analysis were recruited for participation in EMAS. Details concerning the study design and recruitment have been described previously (24). Briefly, men were recruited from population-based sampling frames in 8 centers: Florence (Italy), Leuven (Belgium), Łódź (Poland), Malmö (Sweden), Manchester (United Kingdom), Santiago de Compostela (Spain), Szeged (Hungary), and Tartu (Estonia). Stratified random sampling was used with the aim of recruiting equal numbers of men in each of 4 10-year age bands: 40–49, 50–59, 60–69, and 70–79 years. Subjects were invited by letter to complete a postal questionnaire and attend for an interviewer-assisted questionnaire, clinical assessments and a fasting blood sample. The overall response rate was 45%. Ethical approval for the study was obtained in accordance with local institutional requirements in each center. All subjects provided written informed consent.
Study questionnaires and clinical data

The postal questionnaire included questions concerning current smoking, alcohol consumption in the previous year (response set = every day/5–6 days per week/3–4 days per week/1–2 days per week/less than once a week/not at all) and also whether they were currently being treated for a range of medical conditions, which included diabetes and prostate disease. The interviewer assisted questionnaire comprised the Physical Activity Scale for the Elderly (PASE) and also asked about current medications (25). Subjects also completed the Reubens physical performance test (26). Height was measured to the nearest 1 mm using a stadiometer (Leicester height measure, SECA UK Ltd, Birmingham, United Kingdom) and body weight to the nearest 0.1 kg using an electronic scale (SECA model number 8801321009; SECA UK Ltd). A single fasting morning (before 1000 hours) venous blood sample was obtained from all subjects.

Assessment of 25(OH)D and 1,25(OH)2D3

Serum 25(OH)D levels were determined using a RIA (RIA kit; DiaSorin, Stillwater, Minnesota). Intra- and interassay coefficients of variation (CVs) for 25(OH)D were 11% and 8%, respectively. The detection limit of the RIA kit was 2.0 ng/mL. 1,25-(OH)2D3 was measured by liquid chromatography-tandem mass spectrometry (LC-MS/MS) as a lithium adduct according to the method described by Casetta et al (27). In contrast to this earlier method, methanol instead of acetonitrile was used for protein precipitation of 200 μL serum samples. The injected volume of supernatant was increased from 90 μL to 180 μL and injected on a Shimadzu Prominence HPLC Shimadzu, Kyoto, Japan) (coupled to an AB Sciex API 5500 QTRAP tandem mass spectrometer (Sciex, Warrington, United Kingdom). The use of ultrapure methanol (Fisher; Optima liquid chromatography-mass spectrometry) further helped to increase sensitivity due to reduced ion suppression in the LC-MS/MS interface (28). The 1,25-(OH)2D3 standard dissolved in ethanol was calibrated by measuring the UV absorbance at 264 nm, using a molar absorbance of 18 300. Calibrators (6.25–250 pg/mL) were dissolved in a surrogate matrix containing bovine serum albumin (60 g/L) dissolved in physiological water with the addition of 0.2% serum and a 1,25-(OH)2D3 concentration lower than 10 pg/mL. The internal standard peak area of calibrators or serum samples did not fluctuate more than 20% relative to a water blank. Calibration curves were linear through zero over the entire measuring range from 6.25 to 250 pg/mL. The signal to noise ratio of a 6.25 pg/mL calibrator was greater than 10, allowing the definition of a limit of quantification of less than 6.25 pg/mL. Carryover as measured in a blank after the injection of the highest calibrator level was lower than the limit of detection, the latter defined as 3 times the background noise level. Potential interferences from level was lower than the limit of detection, the latter defined as measured in a blank after the injection of the highest calibrator

Hormone measurements

Measurements of testosterone (T) and estradiol (E2) were carried out by gas chromatography mass spectrometry. SHBG was measured by the Modular E170 platform electrochemiluminescence immunoassay (Roche Diagnostics, Mannheim, Germany). The free and bioavailable (non-SHBG bound) T and E2 levels were derived from total hormone, SHBG, and albumin concentrations using mass action equations and association constants. Further details are described elsewhere (30). In addition, samples were transported in frozen state to a single laboratory for measurement of PTH and IG-F-I (University of Santiago de Compostela). Serum was assayed for PTH using a chemiluminescence immunoassay (Nichols Advantage Bio-Intact PTH assay; Quest Diagnostics, Madison, New Jersey). Interassay CV for PTH was 2.8%. The detection limit of the chemiluminescence immunoassay was 1.6 pg/mL. Serum was assayed for IG-F-I using chemiluminescence as previously described (31).

QUS of the heel

QUS of the left heel was performed with the Sahara clinical sonometer (Hologic, Inc, Waltham, Massachusetts) using a standardized protocol in all centers. Outputs included broadband ultrasound attenuation (BUA; measured in decibels per megahertz) and speed of sound (SOS; measured in meters per second). The in vivo CVs were 2.8% and 0.3% for BUA and SOS, respectively. Repeat measurements were performed on a roving phantom at each of the 8 centers (32). Standardized CVs for within-machine variability ranged by center: for SOS, from 1.0% to 5.6%, and BUA from 0.7% to 2.7%. Standardized CVs for between-machine variability were 4.8% for BUA and 9.7% for SOS (32).

Dual-energy x-ray absorptiometry

Areal bone mineral density (BMDa) scans were carried out in the Manchester and Leuven subsets of EMAS (n = 676). Both sites used DXA QDR 4500A devices from the same manufacturer (Hologic, Inc). BMDa was measured at the lumbar spine (L1 to L4) and proximal femur (total region). The precision errors in Leuven were 0.57% and 0.56% at the lumbar spine and total femur region, respectively. In Manchester, these precision errors were 0.97% and 0.97%, respectively. Both devices were cross-calibrated with the European spine phantom (33).

Bone marker measurements

To assess bone resorption, serum β-C-terminal cross-linked telopeptide (β-c-CTX) was measured on the Elecsys 2010 automated analyzer (Roche Diagnostics GmbH) as previously described (34). The intraassay CV evaluated by repeated measurements of several serum samples was less than 5.0%. The detection limit was 10 pg/mL. To evaluate bone formation, measurements were performed on the Elecsys 2010 with a 2-site assay using monoclonal antibodies raised against intact human N-terminal propeptide of type 1 procollagen (PINP) purified from human amniotic fluid. The interassay CV was less than 3.0% and the lower detection limit less than 5 ng/mL.

Analysis

The association between 1,25(OH)2D and 25(OH)D as well as 1,25(OH)2D, 25(OH)D, and PTH was initially assessed visually using scatter plots and superimposing linear lines and lo-
cally weighted scatter plot smooth curves. The strength of the associations was then determined using linear regression after adjusting for age and center. For ease of interpretation and comparison, 1,25(OH)₂D, 25(OH)D, and PTH were standardized into Z scores (per SD). These variables were also categorized into quintiles to assess the potential threshold effects.

The association between 1,25(OH)₂D, 25(OH)D, PTH, and factors that could potentially confound associations with bone parameters were assessed using linear regression adjusting for age and center. These factors included height (centimeters), weight (kilograms), body mass index (BMI) (kilograms per square meter), PASE score (per 100), time to walk 50 feet (seconds), smoking (percentage), alcohol consumption (categorized by number of days consumed alcohol), and, after standardizing to Z scores, serum calcium, creatinine, total and free T, total and free E₂, SHBG, and IGF-I. Multivariable linear regression was then used to determine the association between 1,25(OH)₂D, 25(OH)D, PTH, and QUS parameters (BUA and SOS), DXA (total hip and lumbar spine), and bone turnover parameters (PINP and β-cTX) with the bone measures as dependent variables, adjusting for age, center, season of measurement, and factors found to be associated with the bone outcomes in the previous analysis. All continuous variables were standardized into Z scores (per SD).

To assess the influence of the combination of 1,25(OH)₂D and 25(OH)D on bone health, subjects were categorized into 4 groups: 1, normal 25(OH)D and 1,25(OH)₂D; 2, normal 25(OH)D and high 1,25(OH)₂D; 3, low 25(OH)D and normal 1,25(OH)₂D; and 4, low 25(OH)D and high 1,25(OH)₂D. Low 25(OH)D was defined as those in the lowest tertile of 25(OH)D (<17.7 ng/mL) and high 1,25(OH)₂D was defined as those in the highest tertile of 1,25(OH)₂D (>64.6 pg/mL). Tertiles were chosen because it provided greater statistical power than quintiles, although broadly similar results were obtained when subjects were categorized using quintiles. A similar categorization was used to assess the combination of low 25(OH)D and high PTH. These models included age, center, season of measurement, and factors found to be associated with the bone outcomes. Results of all linear regression analyses are expressed as standardized β-coefficients and 95% confidence intervals (CIs). Statistical analysis was performed using STATA version 9.2 (http://www.stata.com).

Results

Subjects

A total of 2783 men with a mean age of 60.0 years (SD 11.0) had complete 1,25(OH)₂D, 25(OH)D, QUS, and bone marker data. Characteristics of the subjects are shown in Table 1. Mean BMI was 27.6 kg/m². A little more than one fifth of the subjects reported that they currently smoke, whereas 56% of the men reported consuming alcohol on at least 1 day per week, 4% reported currently taking corticosteroids, and 0.6% was on calcium and/or vitamin D supplementation. Mean 1,25(OH)₂D was 59.3 pg/mL (SD 16.5), 25(OH)D 24.4 ng/mL (SD 12.4), and PTH 28.4 pg/mL (SD 12.1). As expected, there was some variation in 1,25(OH)₂D and 25(OH)D levels according to the season in which they were measured (Fig. 1). The highest 25(OH)D was observed in the summer and autumn (mean 29.6 and 29.9 ng/mL, respectively) and the lowest in the winter and spring months (mean 20.9 and 20.4 ng/mL, respectively). Levels of 1,25(OH)₂D followed a similar pattern. In addition, there was significant variation in 1,25(OH)₂D, 25(OH)D, and PTH by center (Supplemental Table 1, published on The Endocrine Society’s Journals Online web site at http://jcem.endojournals.org); however, there did not appear to be any trend toward decreasing levels 25(OH)D with increasing latitude.

Association between 1,25(OH)₂D, 25(OH)D, and PTH

1,25(OH)₂D was positively correlated with 25(OH)D (β-coefficient = 0.457 pg/mL; P < .001) (Fig. 2A). The association persisted after adjustment for age and center, and there was no evidence of threshold effects when 25(OH)D was categorized into quintiles or an interaction with PTH when PTH was categorized into quintiles (data not shown). There was a modest correlation between 1,25(OH)₂D and PTH (β = −.060 pg/mL; P = .021) (Fig. 2B), which was attenuated after adjustment for age and center. 25(OH)D was negatively associated with PTH (β = −.194 ng/mL; P < .001) (Fig. 2C). This relationship

### Table 1. Subject Characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Subjects (n = 2783)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at interview, y</td>
<td>60.0 (11.0)</td>
</tr>
<tr>
<td>Height, cm</td>
<td>173.5 (7.4)</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>83.3 (13.8)</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>27.6 (4.0)</td>
</tr>
<tr>
<td>PASE score (0–1100)</td>
<td>193.2 (90.2)</td>
</tr>
<tr>
<td>1,25(OH)₂D, pg/mL</td>
<td>59.3 (16.5)</td>
</tr>
<tr>
<td>25(OH)D, ng/mL</td>
<td>24.4 (12.4)</td>
</tr>
<tr>
<td>PTH, pg/mL</td>
<td>28.4 (12.1)</td>
</tr>
<tr>
<td>Creatinine, μmol/L</td>
<td>90.9 (16.5)</td>
</tr>
<tr>
<td>Calcium, mmol/L</td>
<td>2.4 (0.1)</td>
</tr>
<tr>
<td>T, nmol/L</td>
<td>16.4 (6.0)</td>
</tr>
<tr>
<td>Free T, pmol/L</td>
<td>288.9 (88.4)</td>
</tr>
<tr>
<td>E₂, pmol/L</td>
<td>73.5 (24.7)</td>
</tr>
<tr>
<td>Free E₂, pmol/L</td>
<td>1.3 (0.4)</td>
</tr>
<tr>
<td>SHBG, nmol/L</td>
<td>42.9 (19.8)</td>
</tr>
<tr>
<td>IGF-I, ng/mL</td>
<td>132.2 (43.1)</td>
</tr>
<tr>
<td>QUS</td>
<td>80.4 (18.9)</td>
</tr>
<tr>
<td>BUA, dB/MHz</td>
<td>1550.1 (34.2)</td>
</tr>
<tr>
<td>SOS, m/s</td>
<td>1,018 (0.145)</td>
</tr>
<tr>
<td>DXA</td>
<td>1,066 (0.182)</td>
</tr>
<tr>
<td>Total hip, g/cm²</td>
<td>42.4 (20.8)</td>
</tr>
<tr>
<td>Lumbar spine, g/cm²</td>
<td>360.6 (182.4)</td>
</tr>
<tr>
<td>Bone markers</td>
<td>21.1%</td>
</tr>
<tr>
<td>Current smokers</td>
<td>55.5%</td>
</tr>
<tr>
<td>Alcohol consumption</td>
<td>3.6%</td>
</tr>
<tr>
<td>Taking corticosteroids</td>
<td>0.6%</td>
</tr>
</tbody>
</table>

* More than 1 d/wk.
persisted after adjustment for age and center, with no evidence of threshold effects. When an interaction with 1,25(OH)\(_2\)D was explored, the negative association between 25(OH)D and PTH was more marked in subjects in the highest quintile of 1,25(OH)\(_2\)D compared with those in the lowest quintile (β for difference in slope = −0.140; \(P = 0.007\)). Further adjustment for serum calcium levels had no influence on the results.

Association between 1,25(OH)\(_2\)D, 25(OH)D, and PTH and age, anthropometric, lifestyle, and hormonal factors

The association between 1,25(OH)\(_2\)D, 25(OH)D, and PTH and age, anthropometric, lifestyle, hormonal, and biochemical factors are shown in Table 2. 1,25(OH)\(_2\)D decreased and PTH increased with age, but there was no association between 25(OH)D and age. 1,25(OH)\(_2\)D was

Figure 1. 1,25(OH)\(_2\)D (A) and 25(OH)D (B) levels by month of measurement. Values are mean and 95% CI.

Figure 2. Association between 1,25(OH)\(_2\)D and 25(OH)D (A), 25(OH)D and PTH (B), and 1,25(OH)\(_2\)D and PTH (C). The solid lines represent the linear relationship, and the dashed lines represent locally weighted scatterplot smoothing (LOWESS).
associated with higher levels of both markers of bone turnover (Table 3). Higher 1,25(OH)₂D was associated with lower QUS parameters at the heel and DXA BMDₐ at the lumbar spine (Table 4). Similar results were observed for QUS BUA and SOS, so only the results for SOS are presented here. When categorized into quintiles, those in the highest (vs lowest) quintile of 1,25(OH)₂D had significantly lower SOS and total hip and lumbar spine BMDₐ. However, higher 25(OH)D was associated with higher SOS at the heel and DXA BMDₐ at the total hip and lumbar spine. There was some inconsistency across the categories, although there was no evidence of any threshold effects when 25(OH)D was categorized into quintiles (Table 4). PTH was unrelated to the SOS, but higher PTH levels were associated with lower QUS parameters at both the total hip and lumbar spine (Table 4). Further adjustment for creatinine, serum calcium, and total T made no difference to the 1,25(OH)₂D or 25(OH)D results (data not shown), but further adjustment for serum creatinine, calcium, and total T attenuated the associations between PTH and hip and lumbar spine BMDₐ (data not shown).

When the subjects were categorized by both vitamin D metabolite levels, those in the lowest tertile of 25(OH)D

### Table 2. Association Between 1,25(OH)₂D, 25(OH)D, PTH and Age, Anthropometry, Lifestyle, and Hormonal Factors

<table>
<thead>
<tr>
<th></th>
<th>1,25(OH)₂D (per SD)</th>
<th>25(OH)D (per SD)</th>
<th>PTH (per SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>β-Coefficient (95% CI)</strong>^a</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (y)^b</td>
<td>–0.07 (–0.10, –0.04)^c</td>
<td>0.00 (–0.03, 0.03)</td>
<td>0.014 (0.011, 0.017)^c</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>–0.02 (–0.01, 0.00)^c</td>
<td>0.05 (–0.00, 0.11)</td>
<td>0.005 (–0.001, 0.010)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>–0.08 (–0.11, –0.06)^c</td>
<td>–0.04 (–0.07, –0.02)^d</td>
<td>0.007 (0.004, 0.010)^c</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>–0.02 (–0.03, –0.13)^c</td>
<td>–0.20 (–0.29, –0.12)^c</td>
<td>0.022 (0.013, 0.031)^c</td>
</tr>
<tr>
<td>PASE score (per 100)</td>
<td>0.11 (0.062, 0.158)^c</td>
<td>0.13 (0.086, 0.180)</td>
<td>–0.40 (–0.089, 0.100)</td>
</tr>
<tr>
<td>Time to walk 50 feet (sec)</td>
<td>–0.014 (–0.026, –0.002)^e</td>
<td>–0.027 (–0.039, –0.016)^e</td>
<td>0.010 (–0.003, 0.022)</td>
</tr>
<tr>
<td>Current smoker (yes vs no)</td>
<td>–1.72 (–2.62, –0.83)^c</td>
<td>–2.67 (–3.55, –1.79)^c</td>
<td>–0.81 (–1.72, –0.11)</td>
</tr>
<tr>
<td>Alcohol consumption/wk</td>
<td>–0.189 (–0.308, –0.069)^d</td>
<td>–1.34 (–2.53, –0.16)^e</td>
<td>0.10 (–0.113, 0.132)</td>
</tr>
<tr>
<td>&lt;1 day</td>
<td>–0.047 (–0.153, 0.058)</td>
<td>–0.063 (–0.168, 0.041)</td>
<td>0.060 (–0.048, 0.168)</td>
</tr>
<tr>
<td>1–2 days</td>
<td>Referent</td>
<td>Referent</td>
<td>Referent</td>
</tr>
<tr>
<td>3–4 days</td>
<td>0.027 (–0.100, 0.155)</td>
<td>0.009 (–0.118, 0.136)</td>
<td>0.079 (–0.051, 0.210)</td>
</tr>
<tr>
<td>5–6 days</td>
<td>–0.040 (–0.199, 0.119)</td>
<td>–0.029 (–0.187, 0.128)</td>
<td>0.109 (–0.054, 0.271)</td>
</tr>
<tr>
<td>Every day</td>
<td>0.096 (–0.282, 0.219)</td>
<td>–0.009 (–0.131, 0.114)</td>
<td>–0.025 (–0.151, 0.102)</td>
</tr>
<tr>
<td>Creatinine (per SD)</td>
<td>–0.120 (–0.159, –0.082)^c</td>
<td>0.082 (0.044, 0.121)^c</td>
<td>0.086 (0.046, 0.125)^c</td>
</tr>
<tr>
<td>Calcium (per SD)</td>
<td>0.051 (0.008, 0.094)^a</td>
<td>0.086 (0.043, 0.130)^c</td>
<td>–0.136 (–0.178, –0.093)^c</td>
</tr>
<tr>
<td>Total T (per SD)</td>
<td>0.043 (0.006, 0.079)^a</td>
<td>0.054 (0.018, 0.090)^d</td>
<td>–0.051 (–0.088, –0.013)^d</td>
</tr>
<tr>
<td>Free T (per SD)</td>
<td>0.025 (–0.015, 0.065)</td>
<td>0.062 (0.022, 0.101)^d</td>
<td>–0.015 (–0.056, 0.026)</td>
</tr>
<tr>
<td>Total E₂ (per SD)</td>
<td>0.013 (–0.024, 0.049)</td>
<td>–0.024 (–0.060, 0.012)</td>
<td>0.038 (0.001, 0.075)^a</td>
</tr>
<tr>
<td>Free E₂ (per SD)</td>
<td>–0.009 (–0.046, 0.028)</td>
<td>–0.039 (–0.075, –0.002)^a</td>
<td>0.073 (0.036, 0.111)^c</td>
</tr>
<tr>
<td>SHBG (per SD)</td>
<td>0.028 (–0.010, 0.067)</td>
<td>0.009 (–0.029, 0.047)</td>
<td>–0.058 (–0.097, –0.019)^d</td>
</tr>
<tr>
<td>IGF-I (per SD)</td>
<td>–0.010 (–0.047, 0.028)</td>
<td>0.071 (0.034, 0.108)^c</td>
<td>–0.054 (–0.092, –0.015)^d</td>
</tr>
</tbody>
</table>

^a Adjusted for age and center except where adjusted for center only.
^b Adjusted for center.
^c P < .001.
^d P < .01.
^e P < .05.

### Association between 1,25(OH)₂D, 25(OH)D, PTH, and Bone Health Parameters

After adjustment for age, center, height, weight, PASE score, current smoking, alcohol consumption, and season of measurement, higher levels of 1,25(OH)₂D were associated with higher levels of the bone resorption marker β-cTX (Table 3). 1,25(OH)₂D did not appear to be related to the bone formation marker PINP. In contrast, 25(OH)D was not associated with markers of bone turnover. Higher levels of PTH were associated with higher levels of both markers of bone turnover (Table 3).
and the highest tertile of 1,25(OH)2D had higher β-c-CTX as well as lower SOS at the heel and lower BMDa at the total hip and lumbar spine compared with the subjects in the middle to high tertiles of 25(OH)D and middle to low tertiles of 1,25(OH)2D (Tables 3 and 4).

When the subjects were categorized by 25(OH)D and PTH levels, those in the lowest tertile of 25(OH)D and the highest tertile of PTH levels had higher bone turnover markers and lower heel SOS, hip, and lumbar spine BMDa compared with those in the middle to high tertiles of 25(OH)D and middle to low tertiles of PTH.

Excluding those on antosteoporotic medication or those receiving calcium/vitamin D supplementation made no difference to any of the results.

**Discussion**

In this population-based sample of middle-aged and older European men, 1,25(OH)2D was positively associated with 25(OH)D but not with PTH. 25(OH)D was negatively related to markers of bone turnover and weakly negatively associated with BMDa at the hip. As expected, subjects in the lowest tertile of 25(OH)D and the highest tertile of PTH had higher bone turnover and lower QUS and DXA BMDa.

We observed a significant but modest decline in 1,25(OH)2D, but not 25(OH)D, with age in keeping with some (17–19) but not all studies (20, 21). This implies that

Table 3. Association of 1,25(OH)2D, 25(OH)D, and PTH with Bone Turnover

<table>
<thead>
<tr>
<th>β-Coefficient (95% CI)(^a)</th>
<th>P1NP (per SD)</th>
<th>β-c-CTX (per SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,25(OH)2D (per SD)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1,25(OH)2D quintiles</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1: &lt;45.6</td>
<td>.017 (.025, .058)</td>
<td>.162 (.124, .201)(^b)</td>
</tr>
<tr>
<td>2: 45.6–54.0</td>
<td>-.006 (.129, .116)</td>
<td>.049 (.064, .163)</td>
</tr>
<tr>
<td>3: 54.1–61.7</td>
<td>.013 (.111, .138)</td>
<td>.167 (.052, .282)(^c)</td>
</tr>
<tr>
<td>4: 61.8–72.2</td>
<td>.089 (.037, .216)</td>
<td>.288 (.170, .405)(^b)</td>
</tr>
<tr>
<td>5: &gt;72.2</td>
<td>.079 (.050, .209)</td>
<td>.496 (.376, .615)(^b)</td>
</tr>
<tr>
<td>25(OH)D (per SD)</td>
<td>-.039 (.084, .007)</td>
<td>-.029 (.071, .014)</td>
</tr>
<tr>
<td>25(OH)D quintiles</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1: &lt;14.1</td>
<td>Referent</td>
<td>Referent</td>
</tr>
<tr>
<td>2: 14.1–19.4</td>
<td>-.075 (.199, .048)</td>
<td>-.083 (.199, .033)</td>
</tr>
<tr>
<td>3: 19.5–25.3</td>
<td>.011 (.115, .138)</td>
<td>-.012 (.131, .107)</td>
</tr>
<tr>
<td>4: 25.4–33.7</td>
<td>-.080 (.211, .051)</td>
<td>-.112 (.235, .011)</td>
</tr>
<tr>
<td>5: &gt;33.7</td>
<td>-.097 (.238, .044)</td>
<td>-.090 (.222, .043)</td>
</tr>
<tr>
<td>Vitamin D categories</td>
<td></td>
<td></td>
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<tr>
<td>Mid- or highest tertile 25(OH)D/mid- or lowest tertile 1,25(OH)2D</td>
<td>Referent</td>
<td>Referent</td>
</tr>
<tr>
<td>Mid- or highest tertile 25(OH)D/highest tertile 1,25(OH)2D</td>
<td>.047 (.054, .148)</td>
<td>.280 (.186, .374)(^b)</td>
</tr>
<tr>
<td>Lowest tertile 25(OH)D/mid- or lowest tertile 1,25(OH)2D</td>
<td>-.005 (.108, .099)</td>
<td>.055 (.041, .151)</td>
</tr>
<tr>
<td>Lowest tertile 25(OH)D/highest tertile 1,25(OH)2D</td>
<td>.114 (.057, .286)</td>
<td>.453 (.294, .612)(^b)</td>
</tr>
<tr>
<td>PTH (per SD)</td>
<td>.121 (.081, .160)(^b)</td>
<td>.196 (.159, .233)(^b)</td>
</tr>
<tr>
<td>PTH quintiles</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1: &lt;18.84</td>
<td>Referent</td>
<td>Referent</td>
</tr>
<tr>
<td>2: 18.84–23.89</td>
<td>.094 (.029, .216)</td>
<td>.097 (.018, .211)</td>
</tr>
<tr>
<td>3: 23.90–29.11</td>
<td>.191 (.068, .314)(^b)</td>
<td>.222 (.107, .336)(^b)</td>
</tr>
<tr>
<td>4: 29.12–36.31</td>
<td>.266 (.141, .392)(^b)</td>
<td>.316 (.199, .434)(^b)</td>
</tr>
<tr>
<td>5: &gt;36.31</td>
<td>.359 (.234, .485)(^b)</td>
<td>.527 (.410, .644)(^b)</td>
</tr>
<tr>
<td>25(OH)D/PTH categories</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mid- or highest tertile 25(OH)D/mid- or lowest tertile PTH</td>
<td>Referent</td>
<td>Referent</td>
</tr>
<tr>
<td>Mid- or highest tertile 25(OH)D/highest tertile PTH</td>
<td>.203 (.098, .308)(^b)</td>
<td>.276 (.178, .374)(^b)</td>
</tr>
<tr>
<td>Lowest tertile 25(OH)D/mid- or lowest tertile PTH</td>
<td>-.053 (.163, .057)</td>
<td>-.076 (.179, .026)</td>
</tr>
<tr>
<td>Lowest tertile 25(OH)D/highest tertile PTH</td>
<td>.181 (.057, .305)(^b)</td>
<td>.306 (.191, .422)(^b)</td>
</tr>
</tbody>
</table>

\(^a\) Adjusted for age, center, height, weight, PASE score, current smoking, alcohol consumption, and season of measurement.

\(^b\) \(P < .001\).

\(^c\) \(P < .01\).
renal capacity to synthesize 1,25(OH)₂D, in addition to 25(OH)D production in the skin in response to sunlight, may be relatively well conserved, even in elderly community-dwelling men. Sunlight exposure, however, also appeared to have an influence on serum 1,25(OH)₂D as reflected by our observation of seasonal variation in 1,25(OH)₂D levels very similar to that of 25(OH)D. Although the seasonal variation of serum 25(OH)D levels is well established (35, 36), the influence of season on 1,25(OH)₂D has been a matter of debate in the literature (18–20, 37), with some studies reporting seasonal differences in 25(OH)D-deficient subjects only (1, 37), which is consistent with the endocrinological principles of negative feedback. We observed seasonal variation in 1,25(OH)₂D at all levels of 25(OH)D, including in men who were 25(OH)D replete (data not shown).

In our study, as in others (17–19), 1,25(OH)₂D was positively associated with 25(OH)D, also in agreement

<table>
<thead>
<tr>
<th>Table 4. Association of 1,25(OH)₂D, 25(OH)D and PTH With QUS SOS and DXA-Assessed BMDₐ</th>
<th>β-Coefficient (95% CI)ᵃ</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>QUS SOS (per SD)</td>
</tr>
<tr>
<td>1,25(OH)₂D (per SD)</td>
<td>-0.077 (-0.116, -0.038)ᵇ</td>
</tr>
<tr>
<td>1,25(OH)₂D quintiles 1: &lt;45.6</td>
<td>Referent</td>
</tr>
<tr>
<td>2: 45.6–54.0</td>
<td>-0.022 (-0.137, 0.094)</td>
</tr>
<tr>
<td>3: 54.1–61.7</td>
<td>-0.047 (-0.163, 0.070)</td>
</tr>
<tr>
<td>4: 61.8–72.2</td>
<td>-0.080 (-0.199, 0.039)</td>
</tr>
<tr>
<td>5: &gt;72.2</td>
<td>-0.212 (-0.333, -0.090)ᶜ</td>
</tr>
<tr>
<td>25(OH)D (per SD)</td>
<td>0.073 (0.031, 0.116)ᶜ</td>
</tr>
<tr>
<td>25(OH)D quintiles 1: &lt;14.1</td>
<td>Referent</td>
</tr>
<tr>
<td>2: 14.1–19.4</td>
<td>0.046 (-0.071, 0.162)</td>
</tr>
<tr>
<td>3: 19.5–25.3</td>
<td>0.112 (-0.007, 0.232)</td>
</tr>
<tr>
<td>4: 25.4–33.7</td>
<td>0.099 (-0.024, 0.222)</td>
</tr>
<tr>
<td>5: &gt;33.7</td>
<td>0.159 (0.026, 0.291)ᵈ</td>
</tr>
<tr>
<td>Vitamin D categories</td>
<td></td>
</tr>
<tr>
<td>Mid- or highest tertile 25(OH)D/mid- or lowest tertile 1,25(OH)₂D</td>
<td>Referent</td>
</tr>
<tr>
<td>Mid- or highest tertile 25(OH)D/highest tertile 1,25(OH)₂D</td>
<td>-0.156 (-0.251, -0.061)ᶜ</td>
</tr>
<tr>
<td>Lowest tertile 25(OH)D/mid- or lowest tertile 1,25(OH)₂D</td>
<td>-0.126 (-0.223, -0.029)ᵈ</td>
</tr>
<tr>
<td>Lowest tertile 25(OH)D/highest tertile 1,25(OH)₂D</td>
<td>-0.375 (-0.536, -0.214)ᵇ</td>
</tr>
<tr>
<td>PTH (per SD)</td>
<td>-0.023 (-0.061, 0.015)</td>
</tr>
<tr>
<td>PTH quintiles 1: &lt;18.84</td>
<td>Referent</td>
</tr>
<tr>
<td>2: 18.84–23.89</td>
<td>-0.046 (-0.164, 0.072)</td>
</tr>
<tr>
<td>3: 23.90–29.11</td>
<td>0.006 (-0.112, 0.124)</td>
</tr>
<tr>
<td>4: 29.12–36.31</td>
<td>-0.044 (-0.164, 0.077)</td>
</tr>
<tr>
<td>5: &gt;36.31</td>
<td>-0.066 (-0.186, 0.054)</td>
</tr>
<tr>
<td>25(OH)D/PTH categories</td>
<td></td>
</tr>
<tr>
<td>Mid- or highest tertile 25(OH)D/mid- or lowest tertile PTH</td>
<td>Referent</td>
</tr>
<tr>
<td>Mid- or highest tertile 25(OH)D/highest tertile PTH</td>
<td>-0.024 (-0.125, 0.076)</td>
</tr>
<tr>
<td>Lowest tertile 25(OH)D/mid or lowest tertile PTH</td>
<td>-0.096 (-0.201, 0.009)</td>
</tr>
<tr>
<td>Lowest tertile 25(OH)D/highest tertile PTH</td>
<td>-0.148 (-0.266, -0.030)ᵈ</td>
</tr>
</tbody>
</table>

Highest tertile of 1,25(OH)₂D is greater than 64.6 pg/mL, lowest tertile of 25(OH)D is less than 17.7 ng/mL, and highest tertile of PTH is greater than 31.20 pg/mL.

ᵃ Adjusted for age, center, height, weight, PASE score, current smoking, alcohol consumption, and season of measurement.
ᵇ P < .001.
ᶜ P < .01.
ᵈ P < .05.
with the substrate-dependent nature of 1,25(OH)₂D synthesis. However, only approximately 12% of the variation of 1,25(OH)₂D was explained by 25(OH)D, implying that other factors such as diet (calcium and phosphate intake), serum calcium and phosphate concentrations, immobility, and renal function as well as genetic background may also determine 1,25(OH)₂D levels (12). We observed differences in the levels of both 1,25(OH)₂D and, as previously reported (38), 25(OH)D between European centers. These differences were, however, not associated with latitude, and no other specific patterns emerged, with some centers having low 25(OH)D but high 1,25(OH)₂D, whereas others had both high 25(OH)D and 1,25(OH)₂D, providing further evidence that many factors determine vitamin D status at a population level.

We found only a modest correlation between 1,25(OH)₂D and PTH that was attenuated by adjustment for other factors. Previous studies are also discordant, with some finding an association (17, 18) and others not (19). The mechanism for a lack of association is unclear, however, because PTH is considered the major driver of bone resorption in men with low 25(OH)D. The absence of a strong 1,25(OH)₂D-PTH relationship is interesting because the rise of PTH in response to 25(OH)D is often used to define a threshold of serum 25(OH)D. We observed a relationship between 25(OH)D and PTH in keeping with several studies (11, 39, 40).

This is the first study to examine the association between 1,25(OH)₂D and bone turnover. Serum 1,25(OH)₂D was positively associated with the bone resorption marker β-cTX. This higher rate of bone turnover did not, however, translate into lower QUS/DXA parameters across the physiological range of 1,25(OH)₂D: only the highest concentrations of 1,25(OH)₂D (above 72 pg/mL) were associated with lower QUS/DXA parameters. These findings are consistent with the notion that 1,25(OH)₂D in addition to its well-established stimulatory effect on intestinal calcium absorption in response to calcium intake, may also increase bone resorption. Indeed, recent data from in vitro and animal studies suggest that 1,25(OH)₂D may have a direct effect on osteoblasts and hence bone resorption because of its well-established interaction with the receptor activator of nuclear factor-κB/receptor activator of nuclear factor-κB ligand signaling pathway (13, 14). The observation of an association between 1,25(OH)₂D and bone resorption in this cohort may indeed reflect a physiological adaptive mechanism to changes in calcium status, which appears independent of PTH and leads to bone loss only in men with the highest 1,25(OH)₂D levels. We did not observe an association between 25(OH)D and bone turnover, in contrast to a Dutch study of older men and women (11). Although this was slightly surprising, given the relationship between 25(OH)D and PTH, it is possible that the previously observed threshold effect between 25(OH)D and bone turnover markers may not apply to healthy middle-aged and older men.

Data on 1,25(OH)₂D and bone health are scarce and conflicting. A study of 62 healthy men aged 30–92 years found no association between 1,25(OH)₂D and radial or vertebral bone mineral content (21). The Study of Osteoporotic Fractures Research Group provided evidence to suggest that the risk of hip fracture increased by a factor of 2.1 (95% CI 1.2–3.5) in postmenopausal women with low serum 1,25(OH)₂D [≥23 pg/mL (55 pmol/L)] (22); however, these conclusions were based on a relatively small case-control analysis using a Study of Osteoporotic Fractures subset of 133 women who subsequently had hip fractures. Another study, which included men, found lower 1,25(OH)₂D levels in hip fracture patients compared to controls (23). In contrast, the association we observed between 25(OH)D and QUS/DXA parameters has been well documented (2–11), although whether this reflects a causal relationship or merely the fact that 25(OH)D is an excellent marker of general health is still a matter for debate. Our observation of a lack of association between 25(OH)D and markers of bone turnover is also in keeping with some (6) but not all studies (11).

The influence of PTH on bone is well established (1, 41, 42). There are, however, few studies examining the relationship between PTH and bone turnover in men, but our observation, which we have previously reported (34), of a positive association in accord with data from a study of community-dwelling French men aged 55–85 years (6). Our observation of a weak association with BMDa at the hip is also concordant with some other community-based studies of men (3, 5, 6, 42).

What are the implications of these data? These results contribute to the understanding of the influence of 1,25(OH)₂D on bone health in middle-aged and elderly men. As far as we are aware, this is the first population-based study to show that high levels of 1,25(OH)₂D are associated with poorer bone health. Men in the highest tertile of 1,25(OH)₂D and lowest tertile of 25(OH)D had a lumbar spine BMDa almost 1 SD lower, which could equate to a 2-fold increase in risk of fracture (43). A possible explanation for this is that low 25(OH)D (and consequently poorer bone health) is being compensated for by higher PTH, which in turn increases 1,25(OH)₂D levels.

Our study has a number of advantages. It is large and population based and used standardized methods in assessment of QUS, DXA, vitamin D metabolites, PTH, and lifestyle and other characteristics. In addition, we have previously described a new, highly accurate LC-MS/MS method for measuring 1,25(OH)₂D (27) and in this study
examine its performance in a community-based sample for the first time. The addition of lithium salt conjugated favorably with 1,25(OH)2D3, thus increasing its ability to be ionized and measured by the mass spectrometer. This enabled accurate measurement of low concentrations of 1,25(OH)2D in a large number of samples using only 200 μL of serum without the need for time-consuming derivatization.

There are, however, a number of limitations to be considered when interpreting the results. The overall response rate for participation was 45%. It is possible that those invited but who did not take part may have differed with respect to levels of the bone health measurements and vitamin D/PTH than those who took part, and therefore, the data concerning the absolute levels of these parameters need to be interpreted with caution. Any factors influencing participation, however, are unlikely to have influenced the results of the analysis, which was based on an internal comparison of those who participated. This study, like most epidemiological studies, was based on a single assay of 1,25(OH)2D/25(OH)D and PTH levels. The epiforms of 1,25(OH)2D could not be differentiated using our LC-MS/MS method. Some measurement error for serum PTH may have occurred despite the use of morning fasting samples that might not have fully corrected for the diurnal variation of PTH (44). This would have tended to reduce the chances of finding associations between 1,25(OH)2D/25(OH)D, PTH, and BMD rather than produce spurious associations. We did not have accurate data on dietary calcium, dietary/serum phosphate, or any other markers of 1α-hydroxylase activity, which could influence serum 1,25(OH)2D levels. Given the cross-sectional design of the study, it is not possible to determine the temporal or causal nature of the observed relationships. Finally, the study was based on assessment of middle-aged and older European men and extrapolation beyond this group should be undertaken with caution.

In summary, in this population sample of middle-aged and older European men, higher 1,25(OH)2D2 levels were associated with higher bone turnover and poorer bone health despite also being modestly associated with higher 25(OH)D. A combination of high 1,25(OH)2D and low 25(OH)D was associated with the poorest bone health.

Acknowledgments

We thank the men who participated in the 8 countries, the research/nursing staff in the 8 centers: C. Pott, Manchester; E. Wouters, Leuven; M. Nilsson, Malmö; M. del Mar Fernandez, Santiago de Compostela; M. Jedrzejowska, Łódź; H.-M. Tabo, Tartu; A. Heredi, Szeged for their data collection; C. Moseley, Manchester, for data entry and project coordination; and M. Machin, Manchester, for preparing the DXA data. K.A.W. is a senior research scientist working within the Nutrition and Bone Health Core Program at the Medial Research Council Human Nutrition Research, funded by the UK Medical Research Council (Grant U105960371). D.V. is a senior clinical investigator supported by the Clinical Research Fund of the University Hospitals Leuven, Belgium. S.B. is senior clinical investigator of the Fund for Scientific Research-Flanders, Belgium (F.W.O.-Vlaanderen) and holder of the Leuven University Chair in Gerontology and Geriatrics. The EMAS Study Group includes the following: Florence (Gianni Forti, Luisa Petrone, Giovanni Corona); Leuven (Dirk Vanderschueren, Steven Boonen, Herman Borghs); Łódź (Krzysztof Kula, Jolanta Slowikowska-Hilczer, Renata Walczak-Jedrzejowska); London (Ippo Huhtaniemi); Malmö (Alexanders Gwerman); Manchester (Frederick Wu, Alan Silman, Terence O’Neill, Joseph Finn, Philip Steer, Abdelouahid Tajar, David Lee, Stephen Pye); Santiago (Felipe Casanueva, Mary Lange, Ana I Castro); Szeged (Gyorgy Bartfai, Imre Földesi, Imre Fejes); Tartu (Margus Punab, Paul Korovitz); and Turku (Min Jiang).

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The European Male Aging Study is funded by the Commission of the European Communities Fifth Framework Program, Quality of Life and Management of Living Resources, Grant QLK6-CT-2001-00258 and supported by Arthritis Research UK. The principal investigator of EMAS is Professor Frederick Wu, MD, Department of Endocrinology, Manchester Royal Infirmary, Manchester, United Kingdom.

Disclosure Summary: All authors have nothing to disclose.

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8. Araujo AB, Travison TG, Esche GR, Holick MF, Chen TC, McKin-
Publication 5: Influence of lifestyle factors on quantitative heel ultrasound measurements in middle-aged and elderly men
Influence of Lifestyle Factors on Quantitative Heel Ultrasound Measurements in Middle-Aged and Elderly Men

Stephen R. Pye · Vinodh Devakumar · Steven Boonen · Herman Borghs · Dirk Vanderschueren · Judith E. Adams · Kate A. Ward · Gyorgy Bartfai · Felipe F. Casanueva · Joseph D. Finn · Gianni Forti · Aleksander Giwercman · Thang S. Han · Ilpo T. Huhtaniemi · Krzysztof Kula · Michael E. J. Lean · Neil Pendleton · Margus Punab · Alan J. Silman · Frederick C. W. Wu · Terence W. O’Neill · EMAS Study Group

Received: 11 March 2009 / Accepted: 3 October 2009 / Published online: 19 January 2010
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Abstract
We examined the distribution of quantitative heel ultrasound (QUS) parameters in population samples of European men and looked at the influence of lifestyle factors on the occurrence of these parameters. Men aged between 40 and 79 years were recruited from eight European centers and invited to attend for an interviewer-assisted questionnaire, assessment of physical performance, and quantitative ultrasound (QUS) of the calcaneus (Hologic; Sahara). The relationships between QUS parameters and lifestyle variables were assessed using linear regression with adjustments for age, center, and weight. Three thousand two hundred fifty-eight men, mean age 60.0 years, were included in the analysis. A higher PASE score (upper vs. lower tertile) was associated with a higher BUA (β coefficient = 2.44 dB/Mhz), SOS (β = 6.83 m/s), and QUI (β = 3.87). Compared to those who were inactive, those who walked or cycled more than an hour per day had a higher BUA (β = 3.71 dB/Mhz), SOS (β = 6.97 m/s), and QUI (β = 4.50). A longer time to walk 50 ft was linked with a lower BUA (β = 0.62 dB/Mhz), SOS (β = -1.06 m/s), and QUI (β = -0.69). Smoking was associated with a reduction in BUA, SOS, and

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There was a U-shaped association with frequency of alcohol consumption. Modification of lifestyle, including increasing physical activity and stopping smoking, may help optimize bone strength and reduce the risk of fracture in middle-aged and elderly European men.

Keywords Epidemiology · Ultrasound · Bone mineral density · Risk factors · Exercise

Quantitative ultrasound (QUS) measurements at the heel have been shown to be associated with risk of spine and nonspine fracture in men and women [1–9]. The strength of prediction has been reported to be at least as strong as that for bone mineral density (BMD) measurements assessed using dual-energy X-ray absorptiometry (DXA) and to be independent of DXA BMD. There is evidence that fracture rates in men and women vary across Europe, with higher rates in northern than in southern Europe [10, 11]. Whether or not such geographic variation can be explained by variation in the level of ultrasound parameters in these populations is unknown. Furthermore, compared to data using DXA, relatively less is known about the lifestyle factors which influence heel ultrasound parameters in men. There is some evidence that physical activity and smoking may influence heel ultrasound broadband ultrasound attenuation (BUA) and speed of sound (SOS) in men, however, the findings are not always consistent [12–20]. Such data are important: knowledge of risk factors is the first step in the development of effective population wide strategies to optimize bone health.

The European Male Ageing Study is a multicenter population-based study of aging in men aged 40 to 79 years. We used data from the study to determine the distribution of the ultrasound parameters BUA, SOS, and the derived quantitative ultrasound index (QUI) across different European populations and to explore the association between lifestyle factors and these parameters.

Methods

Subjects

The subjects included in this analysis were recruited for participation in the European Male Ageing Study. Men were recruited from population-based sampling frames at eight centers: Florence (Italy), Leuven (Belgium), Lodz (Poland), Malmo (Sweden), Manchester (UK) Santiago del Compostella (Spain), Szeged (Hungary), and Tartu (Estonia). Details regarding recruitment, response rates, and assessments have been described previously [21]. Participating centers were selected to provide geographical and socioeconomic diversity within Europe and facilities to perform epidemiological surveys. Stratified random sampling was performed at each center, with the aim of recruiting 100 men in each of four 10-year age bands: 40–49, 50–59, 60–69, and 70–79 years. Subjects were invited by letter to complete a postal questionnaire and attend for an interviewer-assisted questionnaire, assessment of physical performance, and ultrasound of the heel. Subjects were recontacted, usually within 4 weeks, if they did not reply following the first letter. Ethical approval for the study was obtained in accordance with local institutional requirements at each center.

Assessments

The postal questionnaire included questions concerning time spent walking or on a bicycle out of doors each day (response set = none, <30 min, 30 min to 1 h, >1 h); smoking—both ever and currently; and alcohol consumption in the previous year (response set = every day, 5–6 days per week, 3–4 days per week, 1–2 days per week, less than once a week, not at all). There was also a question about prior fracture since the age of 25 years (response set = no, yes, don’t know). The main study questionnaire included the physical activity scale for the elderly (PASE) and the SF36 quality of life questionnaire [22, 23]. Subjects were also asked whether they were currently being treated for any of a list of 14 morbidities: heart conditions, high blood pressure, pituitary disease, testicular disease, chronic
bronchitis, asthma, peptic ulcer, epilepsy, diabetes, liver conditions, kidney conditions, prostate disease, adrenal disease, and thyroid disease. A number of performance measures were undertaken including the Tinetti assessment of balance and gait, which included the time taken to go from sitting to standing position five times and the time taken to walk 50 ft [24, 25]. In addition, height and weight were measured in a standardized fashion.

Quantitative Heel Ultrasound

Quantitative ultrasound of the heel was performed using the Sahara Clinical Sonometer (Hologic, Bedford, MA, USA) using a standardized protocol. Each center used the same machine model, and each calibrated daily with the physical phantom provided by the manufacturer. Outputs included BUA and SOS. In addition, machine-derived parameters were quantitative ultrasound index (QUI), a measure of stiffness \[ \text{QUI} = 0.41(\text{SOS}) + 0.41(\text{BUA}) - 571 \]; and estimated heel BMD \[ \text{eBMD} = 0.002592 \times (\text{BUA} + \text{SOS}) - 3.687 \]. Quality control (QC) was performed at each center as per the manufacturer’s instructions. All QC results were sent to Leuven and compiled and checked for stability throughout the study. To establish the short-term precision of the method in this population, duplicate measurements were performed in 20 randomly selected cohort members at one of the centers (Leuven, Belgium). The in vivo coefficient of variations (CVs) were 2.8% and 0.3% for BUA and SOS, respectively, and 2.3% and 3.4% for QUI and eBMD, respectively. Repeat measurements (10) were performed on a roving phantom at each of the eight centers. Standardized CVs (SCVs) [26] for within-machine variability ranged by center: for SOS, from 1.0% to 5.6%; and for BUA, from 0.7% to 2.7%. SCVs for between machine-variability were 4.8% for BUA and 9.7% for SOS.

Analysis

Descriptive statistics were used to characterize the distribution of the heel ultrasound parameters (BUA, SOS, QUI, and eBMD) by age and center. In the analysis of risk factors PASE and SF36 physical component score (PCS) were categorized into tertiles. We grouped comorbidities by number (none, one, two or more), although we also looked separately at the more frequent individual comorbidities including heart disease, hypertension, cardiovascular disease, bronchitis, diabetes, and prostate disease. Body mass index (BMI) was calculated by dividing weight (kg) by height squared (m²).

Linear regression was used to determine the association between each of the measured ultrasound parameters, and also QUI, and the various putative risk factors (including age) with the ultrasound parameters as the dependent variables. The results are expressed as absolute differences (\( \beta \) coefficients) and 95% confidence intervals (CIs). Adjustments were made initially for age, weight, and center. A multivariable analysis was then performed with statistical models including all the lifestyle factors that were significantly associated with the QUS parameters and age, weight and center. We also looked at the association between prior fracture and the ultrasound parameters. Statistical analysis was performed using STATA version 9.2.

Results

Subjects

A total of 3258 men with a mean age of 60.0 years (SD = 11.0 years) had QUS measurements performed. There were 772 men aged 40–49 years, 874 aged 50–59 years, 816 aged 60–69 years, and 796 aged >70 years. The number of men at each center ranged from 388 to 427. Characteristics of the subjects are reported in Table 1. Mean BMI was 27.6 kg/m² (SD = 4.0 kg/m²); PASE score, 196.2 (SD = 91.7); and SF36 physical score, 50.0 (SD = 8.2). Sixty-five percent of subjects reported walking or cycling for more than 0.5 h per day. 70% reported having

Table 1  Subject characteristics (N = 3258)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at interview (years)</td>
<td>60.0 (11.0)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>173.6 (7.3)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>83.3 (13.7)</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>27.6 (4.0)</td>
</tr>
<tr>
<td>PASE score (0–1100)</td>
<td>196.2 (91.7)</td>
</tr>
<tr>
<td>SF36 Physical score (0–100)</td>
<td>50.0 (8.2)</td>
</tr>
<tr>
<td>Tinetti: time taken to go from sitting to standing (s)</td>
<td>12.7 (4.2)</td>
</tr>
<tr>
<td>PPT: time taken to walk 50 ft (s)</td>
<td>13.6 (3.4)</td>
</tr>
<tr>
<td>Broadband ultrasound attenuation (dB/MHz)</td>
<td>80.2 (19.0)</td>
</tr>
<tr>
<td>Speed of sound (m/s)</td>
<td>1550.7 (34.1)</td>
</tr>
<tr>
<td>Bone mineral density (g/cm²)</td>
<td>0.542 (0.137)</td>
</tr>
<tr>
<td>Quantitative ultrasound index</td>
<td>97.7 (21.2) %</td>
</tr>
<tr>
<td>Walking or cycling for 0.5 h or more/day</td>
<td>65.3</td>
</tr>
<tr>
<td>Ever smoked (yes vs. no)</td>
<td>70.1</td>
</tr>
<tr>
<td>Currently smoke (yes vs. no)</td>
<td>21.0</td>
</tr>
<tr>
<td>Alcohol consumption ≥1 day/week</td>
<td>56.2</td>
</tr>
</tbody>
</table>

Comorbidities

| None                           | 49.8 |
| One                           | 27.7 |
| Two or more                   | 22.6 |

Previous fracture since age 25 (yes vs. no) 25.9
ever smoked, and 21% reported that they currently smoke. Fifty-six percent of the men reported consuming alcohol at least 1 day per week. Twenty-three percent reported two or more comorbidities and 26% reported a previous fracture. Mean BUA was 80.2 dB/MHz; SOS, 1550.7 m/s; and the derived indexes eBMD, 0.542 g/cm²; and QUI, 97.7. Characteristics of the subjects by center are reported in Table 2. There were significant differences in weight, PASE score, SF36 physical score, time taken to go from a sitting to a standing position, time taken to walk 50 ft, time spent walking/cycling, smoking status, alcohol consumption, number of comorbidities, and self-reported previous fracture by center.

Associations with Age, Center, and Previous Fracture

There were significant differences in the measured parameters BUA and SOS, and the derived parameter QUI, by center; see Table 3. BUA was lowest in Szeged, Hungary, and highest in Manchester, UK (70.4 vs. 87.7 dB/MHz), as was QUI (90.4 vs. 104.9). SOS values were lowest in Florence, Italy, and highest in Leuven, Belgium (1542.5 vs. 1561.2 m/s). There was no evidence of any consistent geographic trend toward higher or lower levels in northwestern (Leuven, Malmo, Manchester), southern (Florence, Santiago), or eastern (Lodz, Tartu, Szeged) Europe. The variation by center was similar in those aged above and below 65 years. There was an apparent decline in BUA, SOS, and QUI with age (see Figs. 1, 2, 3), though given the cross-sectional nature of the data, this needs to be interpreted with some caution. There was no evidence of any significant age × center interaction for BUA (p = 0.49), SOS (p = 0.24), or QUI (p = 0.33). After adjustment for age, an increase in weight was significantly associated with an increase in all three QUS parameters: BUA (β coefficient = 0.21 dB/Mhz), SOS (β = 0.17 m/s), and QUI (β = 0.16). Significant center differences in the ultrasound parameters persisted after adjustment for age, weight, and, also, number and type of comorbidities and, also, after further adjustment, for frequency of alcohol intake, smoking, and physical activity. After adjustment for age, weight, and center, those with a history of self-reported fracture since age 25 years had significantly lower ultrasound values compared to those without: BUA (β = −5.4 dB/Mhz), SOS (β = −10.4 m/s), and QUI (β = −6.5).

Association with Lifestyle Factors

The association between SOS, BUA, and QUI and lifestyle factors is reported in Table 4. The major finding was that increasing levels of physical activity were associated with an increase in all three QUS parameters. After adjustment

---

Table 2: Subject characteristics by center

<table>
<thead>
<tr>
<th>Center</th>
<th>Mean (SD)</th>
<th>% ever smoked</th>
<th>% currently smoke</th>
<th>% alcohol consumption</th>
<th>% two or more comorbidities</th>
<th>% previous fracture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leuven</td>
<td>83.3 (13.7)</td>
<td>70.1</td>
<td>21.0</td>
<td>56.2</td>
<td>50.3</td>
<td>25.9</td>
</tr>
<tr>
<td>Malmo</td>
<td>82.1 (13.0)</td>
<td>64.4</td>
<td>17.2</td>
<td>64.7</td>
<td>46.1</td>
<td>22.6</td>
</tr>
<tr>
<td>Manchester</td>
<td>85.4 (13.2)</td>
<td>68.8</td>
<td>16.5</td>
<td>64.4</td>
<td>38.6</td>
<td>21.3</td>
</tr>
<tr>
<td>Lodz</td>
<td>81.1 (13.3)</td>
<td>56.6</td>
<td>25.7</td>
<td>64.7</td>
<td>39.0</td>
<td>36.3</td>
</tr>
<tr>
<td>Szeged</td>
<td>81.1 (12.8)</td>
<td>82.1</td>
<td>12.9</td>
<td>76.7</td>
<td>13.4</td>
<td>13.4</td>
</tr>
<tr>
<td>Tartu</td>
<td>88.5 (14.2)</td>
<td>86.5</td>
<td>14.2</td>
<td>84.0</td>
<td>13.0</td>
<td>13.0</td>
</tr>
<tr>
<td>Florence</td>
<td>91.7 (14.3)</td>
<td>91.7</td>
<td>14.3</td>
<td>91.7</td>
<td>14.3</td>
<td>14.3</td>
</tr>
<tr>
<td>Santiago</td>
<td>841.1 (80.1)</td>
<td>841.1</td>
<td>80.1</td>
<td>841.1</td>
<td>80.1</td>
<td>80.1</td>
</tr>
<tr>
<td>All centers</td>
<td>Overall</td>
<td>83.3 (13.7)</td>
<td>70.1</td>
<td>21.0</td>
<td>56.2</td>
<td>50.3</td>
</tr>
</tbody>
</table>

---

Table 3: QUS measurements by center

<table>
<thead>
<tr>
<th>Center</th>
<th>Mean BUA</th>
<th>Mean SOS</th>
<th>Mean QUI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leuven</td>
<td>83.3 (13.7)</td>
<td>83.3 (13.7)</td>
<td>83.3 (13.7)</td>
</tr>
<tr>
<td>Malmo</td>
<td>82.1 (13.0)</td>
<td>82.1 (13.0)</td>
<td>82.1 (13.0)</td>
</tr>
<tr>
<td>Manchester</td>
<td>85.4 (13.2)</td>
<td>85.4 (13.2)</td>
<td>85.4 (13.2)</td>
</tr>
<tr>
<td>Lodz</td>
<td>81.1 (13.3)</td>
<td>81.1 (13.3)</td>
<td>81.1 (13.3)</td>
</tr>
<tr>
<td>Szeged</td>
<td>81.1 (12.8)</td>
<td>81.1 (12.8)</td>
<td>81.1 (12.8)</td>
</tr>
<tr>
<td>Tartu</td>
<td>88.5 (14.2)</td>
<td>88.5 (14.2)</td>
<td>88.5 (14.2)</td>
</tr>
<tr>
<td>Florence</td>
<td>91.7 (14.3)</td>
<td>91.7 (14.3)</td>
<td>91.7 (14.3)</td>
</tr>
<tr>
<td>Santiago</td>
<td>841.1 (80.1)</td>
<td>841.1 (80.1)</td>
<td>841.1 (80.1)</td>
</tr>
<tr>
<td>All centers</td>
<td>Overall</td>
<td>Overall</td>
<td>Overall</td>
</tr>
</tbody>
</table>

---

Table 4: Association with lifestyle factors

### Lifestyle Factors

<table>
<thead>
<tr>
<th>Factor</th>
<th>B coefficient (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical activity</td>
<td>0.21 (0.18, 0.25)</td>
</tr>
<tr>
<td>Smoking status</td>
<td>-0.17 (0.14, 0.20)</td>
</tr>
<tr>
<td>Alcohol consumption</td>
<td>-0.16 (0.13, 0.19)</td>
</tr>
</tbody>
</table>

---

S. R. Pye et al.: Lifestyle Factors and Quantitative Ultrasound
for age, weight, and center, compared to those in the lowest tertile of PASE score, those in the upper tertile had an increased BUA ($\beta = 2.44$ dB/MHz), SOS ($\beta = 6.83$ m/s), and QUI ($\beta = 3.87$). Similarly, compared to those who did no walking or cycling, those who walked or cycled for more than 1 h per day had an increased BUA ($\beta = 3.71$ dB/MHz), SOS ($\beta = 6.97$ m/s), and QUI ($\beta = 4.50$). A longer time taken to go from sitting to standing (five times) was associated with a reduced BUA ($\beta = -0.19$ dB/MHz), SOS ($\beta = -0.53$ m/s), and QUI ($\beta = -0.29$), as was a longer time taken to walk 50 ft ($\beta = -0.62$ dB/MHz, -1.06 m/s, and -0.69 for BUA, SOS, and QUI, respectively). Compared to those in the lowest tertile of SF36 physical component score, those in the upper tertile had an increased BUA ($\beta = 2.80$ dB/MHz), SOS ($\beta = 5.75$ m/s), and QUI ($\beta = 3.44$). Further adjustment for individual comorbid conditions (heart disease, hypertension, cardiovascular disease, bronchitis, diabetes, prostate disease) or by number of comorbidities did not attenuate the association between QUS parameters and the physical activity measures, suggesting that the association is not explained by the presence of underlying comorbid disease.

Other lifestyle factors were associated with the ultrasound parameters. After adjustment for age, center, and weight, compared to those who have never smoked, those who ever smoked had a lower BUA ($\beta = -4.55$ dB/MHz), SOS ($\beta = -10.32$ m/s), and QUI ($\beta = -0.01$). Similarly, those

Table 3  Quantitative ultrasound parameters by center: mean (SD)

<table>
<thead>
<tr>
<th>Region</th>
<th>Center</th>
<th>n</th>
<th>Broadband attenuation (dB/MHz)</th>
<th>Speed of sound (m/s)</th>
<th>Quantitative ultrasound index</th>
</tr>
</thead>
<tbody>
<tr>
<td>North/West</td>
<td>Leuven</td>
<td>424</td>
<td>82.4 (18.3)</td>
<td>1561.2 (32.9)</td>
<td>102.9 (20.5)</td>
</tr>
<tr>
<td></td>
<td>Malmo</td>
<td>389</td>
<td>81.4 (16.4)</td>
<td>1549.3 (29.8)</td>
<td>97.5 (18.2)</td>
</tr>
<tr>
<td></td>
<td>Manchester</td>
<td>388</td>
<td>87.7 (17.1)</td>
<td>1560.8 (34.5)</td>
<td>104.9 (20.6)</td>
</tr>
<tr>
<td>East</td>
<td>Lodz</td>
<td>403</td>
<td>79.9 (18.4)</td>
<td>1547.1 (32.6)</td>
<td>96.1 (20.3)</td>
</tr>
<tr>
<td></td>
<td>Szeged</td>
<td>425</td>
<td>70.4 (19.0)</td>
<td>1542.7 (30.1)</td>
<td>90.4 (19.7)</td>
</tr>
<tr>
<td></td>
<td>Tartu</td>
<td>397</td>
<td>79.3 (17.9)</td>
<td>1544.5 (33.6)</td>
<td>94.8 (20.7)</td>
</tr>
<tr>
<td>South</td>
<td>Florence</td>
<td>427</td>
<td>77.0 (17.6)</td>
<td>1542.5 (32.6)</td>
<td>93.0 (20.0)</td>
</tr>
<tr>
<td></td>
<td>Santiago</td>
<td>405</td>
<td>84.1 (21.5)</td>
<td>1557.7 (39.5)</td>
<td>102.3 (24.5)</td>
</tr>
</tbody>
</table>

Fig. 1 Association between broadband ultrasound attenuation (BUA) and age

Fig. 2 Association between speed of sound (SOS) and age

Fig. 3 Association between quantitative ultrasound index (QUI) and age
who currently smoked had a lower BUA ($\beta = -4.96$ dB/MHz), SOS ($\beta = -11.44$ m/s), and QUI ($\beta = -6.57$). There was a U-shaped association with frequency of alcohol consumption. Compared to those who drank one or two times per week, those who did not drink at all had a lower BUA ($\beta = -3.19$ dB/MHz), SOS ($\beta = -4.92$ m/s), and QUI ($\beta = -3.43$). Also, compared to those who drank one or two times per week, those who consumed alcohol every day had a lower BUA ($\beta = -3.73$ dB/MHz), SOS ($\beta = -7.63$ m/s), and QUI ($\beta = -4.73$). Further adjustment for individual comorbid conditions (heart disease, hypertension, cardiovascular disease, bronchitis, diabetes, prostate disease) or number of comorbidities did not attenuate the association between QUS parameters and smoking (ever or current) or alcohol consumption.

When all the lifestyle factors that were associated with the ultrasound parameters were included in a multivariable model, BUA remained positively associated with measures of physical activity, including time spent walking or cycling and SF36 physical score, and negatively associated with time taken to walk 50 ft, current smoking, and alcohol consumption (see Table 5). Similarly, SOS and QUI remained positively associated with PASE score, time spent walking or cycling, and SF36 physical score and negatively associated with time taken to walk 50 ft, current smoking, and alcohol consumption.

### Discussion

In this population survey, there was evidence of variation in levels of measured heel ultrasound parameters, BUA, SOS, and also QUI across Europe. The ultrasound parameters decreased with age, with no evidence, however, of

### Table 4  Influence of lifestyle factors on ultrasound parameters (with adjustments for age, center, and weight): $\beta$ coefficient$^a$ (95% CI)

<table>
<thead>
<tr>
<th>Lifestyle Factor</th>
<th>Broadband attenuation (dB/MHz)</th>
<th>Speed of sound (m/s)</th>
<th>Quantitative ultrasound index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ever smoked (yes vs. no)</td>
<td>-4.551 (-5.923, -3.179)$^*$</td>
<td>-10.319 (-12.811, -7.826)$^*$</td>
<td>-6.007 (-7.553, -4.461)$^*$</td>
</tr>
<tr>
<td>Alcohol consumption (days/week)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>-3.186 (-5.292, -1.081)$^*$</td>
<td>-4.923 (-8.770, -1.076)$^*$</td>
<td>-3.427 (-5.809, -1.046)$^*$</td>
</tr>
<tr>
<td>&lt;1</td>
<td>-0.950 (-2.798, 0.898)</td>
<td>-2.029 (-5.406, 1.348)</td>
<td>-1.137 (-3.427, 0.753)</td>
</tr>
<tr>
<td>1–2</td>
<td>Referent</td>
<td>Referent</td>
<td>Referent</td>
</tr>
<tr>
<td>3–4</td>
<td>-2.318 (-4.545, -0.091)$^*$</td>
<td>-4.705 (-8.774, -0.635)$^*$</td>
<td>-2.976 (-5.495, -0.457)$^*$</td>
</tr>
<tr>
<td>5–6</td>
<td>-2.870 (-5.600, -0.140)$^*$</td>
<td>-5.383 (-10.371, -0.395)$^*$</td>
<td>-3.469 (-6.557, -0.382)$^*$</td>
</tr>
<tr>
<td>Every day</td>
<td>-3.730 (-5.876, -1.584)$^*$</td>
<td>-7.631 (-11.551, -3.710)$^*$</td>
<td>-4.733 (-7.159, -2.306)$^*$</td>
</tr>
<tr>
<td>PASE score per 100 (0–1100)</td>
<td>1.547 (0.727, 2.366)$^*$</td>
<td>3.727 (2.237, 5.218)$^*$</td>
<td>2.197 (1.273, 3.121)$^*$</td>
</tr>
<tr>
<td>PASE score tertile</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lower</td>
<td>Referent</td>
<td>Referent</td>
<td>Referent</td>
</tr>
<tr>
<td>Mid</td>
<td>1.983 (0.326, 3.641)$^*$</td>
<td>4.659 (1.645, 7.674)$^*$</td>
<td>2.837 (0.968, 4.705)$^*$</td>
</tr>
<tr>
<td>Upper</td>
<td>2.442 (0.604, 4.281)$^*$</td>
<td>6.829 (3.484, 10.173)$^*$</td>
<td>3.865 (1.792, 5.938)$^*$</td>
</tr>
<tr>
<td>Walking or cycling/day</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>Referent</td>
<td>Referent</td>
<td>Referent</td>
</tr>
<tr>
<td>&lt;0.5 h</td>
<td>3.046 (0.827, 5.264)$^*$</td>
<td>5.298 (1.248, 9.348)$^*$</td>
<td>3.509 (1.002, 6.016)$^*$</td>
</tr>
<tr>
<td>0.5–1 h</td>
<td>3.042 (0.946, 5.138)$^*$</td>
<td>5.835 (2.008, 9.662)$^*$</td>
<td>3.850 (1.481, 6.220)$^*$</td>
</tr>
<tr>
<td>&gt;1 h</td>
<td>3.711 (1.539, 5.883)$^*$</td>
<td>6.972 (3.006, 10.938)$^*$</td>
<td>4.500 (2.045, 6.955)$^*$</td>
</tr>
<tr>
<td>Walking or cycling (&lt;0.5 vs. ≥0.5 h)</td>
<td>1.355 (-0.017, 2.727)</td>
<td>2.890 (0.385, 5.395)$^*$</td>
<td>1.856 (0.305, 3.407)$^*$</td>
</tr>
<tr>
<td>Tinetti: time taken to go from sitting to standing (s)</td>
<td>-0.187 (-0.343, -0.030)$^*$</td>
<td>-0.526 (-0.811, -0.241)$^*$</td>
<td>-0.294 (-0.470, -0.117)$^*$</td>
</tr>
<tr>
<td>PPT: time taken to walk 50 ft (s)</td>
<td>-0.615 (-0.825, -0.404)$^*$</td>
<td>-1.056 (-1.439, -0.672)$^*$</td>
<td>-0.690 (-0.927, -0.452)$^*$</td>
</tr>
<tr>
<td>SF36 physical component score (0–100)</td>
<td>0.261 (0.177, 0.345)$^*$</td>
<td>0.503 (0.350, 0.656)$^*$</td>
<td>0.312 (0.218, 0.407)$^*$</td>
</tr>
<tr>
<td>SF36 physical component score: tertiles</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lower</td>
<td>Referent</td>
<td>Referent</td>
<td>Referent</td>
</tr>
<tr>
<td>Mid</td>
<td>2.995 (1.407, 4.584)$^*$</td>
<td>5.300 (2.409, 8.191)$^*$</td>
<td>3.435 (1.643, 5.227)$^*$</td>
</tr>
<tr>
<td>Upper</td>
<td>2.796 (1.139, 4.452)$^*$</td>
<td>5.754 (2.739, 8.769)$^*$</td>
<td>3.440 (1.571, 5.309)$^*$</td>
</tr>
</tbody>
</table>

$^a$ p < 0.05

$^a$ Adjusted for age, center, and weight
any important age × center interaction. Increased levels of physical activity and physical performance were associated with higher BUA and SOS, while smoking was associated with lower values. There was a U-shaped association with the frequency of alcohol intake.

Our study was population based and used standardized methods in assessment of QUS and of lifestyle and other characteristics. There are, however, limitations which need to be considered when interpreting the results. The overall response rate for participation was 45%. It is possible that those who were invited but did not take part may have differed with respect to levels of the ultrasound parameters. Assessment for possible response bias requires some auxiliary data about nonresponders. In EMAS a sample of nonresponders was contacted by telephone and invited to complete a short survey. Compared to EMAS participants, those who took part in the telephone survey (n = 361) were more likely to be current smokers (33 vs. 21%; p < 0.001).

No differences were found, however, in general health, time spent walking or cycling per day, or proportion who had ever smoked [21]. While some caution is needed in interpreting the data, factors influencing participation are unlikely to have influenced the results of the risk factor analysis, which was based on an internal comparison of those who participated. Questionnaires and other instruments used in the study were translated from English into the seven European languages in which their use was intended. Given concerns that subtle differences in the translation process may have influenced questionnaire responses, the questionnaire was back-translated by language experts from the relevant European language back into English. Given that the subjects were unaware of their bone ultrasound measurement results, any misclassification related to questionnaire response is likely, however, to be random and therefore would tend to reduce the likelihood of finding significant biological associations. There are no published methods for cross-calibration of QUS [27] and the results reported are the data as obtained at each center. Any errors related to measurement, however, are likely to be nondirectional and would tend to reduce the risk of finding significant biological associations. Given the cross-sectional design of the study, it is not possible to determine the temporal nature of the observed relationships, for which prospective data are needed, although it seems unlikely that lower ultrasound parameters would lead to a reduction in levels of physical activity. Finally, the study was based on assessment of middle-aged and elderly European men and extrapolation beyond this group should be undertaken with caution.

In the analyses there was variation in the distribution of the ultrasound parameters across Europe. There was no consistent geographic trend toward higher or lower levels in northern or southern Europe. There is evidence from epidemiological studies that fracture rates are higher in Scandinavia than elsewhere in Europe [10, 11], however, in...
our study the mean BUA and SOS in Malmo, Sweden, were close to the average in these European men. As discussed, however, we did not undertake any cross-calibration and some caution is required in interpreting these data. Our data show a decline in bone ultrasound parameters with age, with no evidence of any important center difference in the rate of decline. Over the four-decade span of those who participated, the average annual decline amounted to 0.16% for BUA, 0.03% for SOS, and 0.23% for QUI. In an observational study of 1138 Spanish men aged 18–99, using the same measurement device, the QUS parameters declined by between 0.08% and 0.41% per year [28]. A decrease in the parameters with age has been observed using other sonometers [17, 19, 29–32].

In our study physical activity was positively associated with the bone ultrasound parameters. The activity measures which we studied, however, primarily assessed the amount or volume of activity rather than the loading, which may be more important to bone health. Most, though not all, studies which have examined the impact of physical activity on heel ultrasound parameters in men suggest a beneficial effect [12–20]. In one of the largest studies, of 4981 men, aged 60–80 years, recalled physical activity was linked with increased QUI as measured using a Lunar Achilles device [17]. Most studies, however, have focused on historical or self-report of physical activity linked with ultrasound measures. In our study we observed an association also with two physical performance measures—the time taken to go from sitting to standing five times and the time to walk 50 ft—with those who took longer to perform these activities having lower ultrasound parameters. It seems likely that these are a proxy for higher levels of physical activity including higher intensity activities and consequent bone loading, though we cannot confirm or refute this [33]. Also, our assessment of bone health was restricted to the heel and it is possible that the effect may differ at other skeletal sites. In women, for example, walking has a greater impact on the calcaneus than the hip and spine [34].

Other lifestyle factors were important in men. Smoking, both ever and current, was linked with a reduction in all ultrasound parameters. Previous studies using both the Sahara device and other sonometers provide somewhat discrepant findings, with some, though not all, reporting a negative association [12, 13, 15–17, 19, 29, 32, 35]. There are fewer data concerning the impact of alcohol consumption, with most suggesting no association [12, 15, 17, 36–40]. In our study we found a U-shaped association between alcohol consumption and the QUS measures, where compared to moderate drinking, both light and heavy drinking were associated with a reduction in QUS. This is consistent with some data from bone mass measurement studies finding that social drinking is associated with beneficial effects on bone mass [41].

What is the potential impact of our findings in relation to fracture occurrence? QUS parameters have been linked with fracture in previous studies in both men and women [1–9]. In our study a self reported history of fracture was associated with a reduction in BUA, SOS, and QUI. Data from a large prospective study showed that the risk of both hip and non-hip fracture increased by a factor of twofold for each unit (SD) change in BUA and SOS [5]. In our study the difference in SOS between those who did and those who did not smoke amounted to an approximate one-third of a standard deviation of the measurement, while for the physical activity scores (PASE and SF36 PCS) the difference in SOS between those in the lowest and those in the highest tertiles of activity was about one-fifth of a standard deviation. Although the risk of fracture attributable to the different exposures is relatively low, given that they are potentially modifiable and common, they would certainly be potential candidates for inclusion in a population strategy for fracture prevention to optimize bone health in middle-age and elderly men, with the ultimate aim of reducing fracture occurrence. Some caution, however, is needed in interpreting the data in relation to physical activity, as physical activity may influence fracture risk by influencing susceptibility to and also risk of falls [33, 42].

In this population survey of European men, QUS parameters declined with age and varied by center across Europe. Lifestyle factors including physical activity, smoking, and alcohol intake influenced bone health.

Acknowledgments The European Male Ageing Study (EMAS) is funded by the Commission of the European Communities Fifth Framework Programme, “Quality of Life and Management of Living Resources,” Grant QLK6-CT-2001-00258, and supported by funding from the U.K. Arthritis Research Campaign. For additional information regarding EMAS, contact Frederick Wu. The authors wish to thank the men of the eight countries who participated; the research/nursing staff at the eight centers—C. Pott (Manchester), E. Wouters, (Leuven), M. Nilsson (Malmo), M. del Mar Fernandez (Santiago de Compostela), M. Jedrzejowska (Lodz), H.-M. Tabo (Tartu), and A. Heredi (Szeged)—for their data collection; and C. Moseley, Manchester, for data entry and project coordination. Dr. Vanderschueren and Dr. Boonen are senior clinical investigators of the Fund for Scientific Research, Flanders, Belgium (F.W.O.-Vlaanderen). Dr. Boonen is holder of the Leuven University Chair in Metabolic Bone Diseases.

References


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S. R. Pye et al.: Lifestyle Factors and Quantitative Ultrasound 219


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Publication 6: Childhood fractures do not predict future fractures: results from the European Prospective Osteoporosis Study
Childhood Fractures Do Not Predict Future Fractures: Results From the European Prospective Osteoporosis Study

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ABSTRACT: Childhood fractures are common. Their clinical relevance to osteoporosis and fractures in later life is unclear. The aim of this study was to determine the predictive risk of childhood fracture on the risk of fracture in later life. Men and women ≥50 yr of age were recruited from population registers for participation in the European Prospective Osteoporosis Study (EPOS). Subjects completed an interviewer administered questionnaire that included questions about previous fractures and the age at which the first of these fractures occurred. Lateral spine radiographs were performed to ascertain prevalent vertebral deformities. Subjects were followed prospectively by postal questionnaire to determine the occurrence of clinical fractures. A subsample of subjects had BMD measurements performed. Cox proportional hazards model was used to determine the predictive risk of childhood fracture between the ages of 8 and 18 yr on the risk of future limb fracture and logistic regression was used to determine the association between reported childhood fractures and prevalent vertebral deformity. A total of 6451 men (mean age, 63.8 yr) and 6936 women (mean age, 63.1 yr) were included in the analysis. Mean follow-up time was 3 yr. Of these, 574 (8.9%) men and 313 (4.5%) women reported a first fracture (any site) between the ages of 8 and 18 yr. A recalled history of any childhood fracture or forearm fracture was not associated with an increased risk of future limb fracture or prevalent vertebral deformity in either men or women. Among the 4807 subjects who had DXA measurements, there was no difference in bone mass among those subjects who had reported a childhood fracture and those who did not. Our data suggest that self-reported previous childhood fracture is not associated with an increased risk of future fracture in men or women.

J Bone Miner Res 2009;24:1314–1318. Published online on February 16, 2009; doi: 10.1359/JBMR.090220

Key words: childhood fracture, prevalent vertebral fracture, incident limb fracture, epidemiology, prospective study

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INTRODUCTION

Childhood fractures are relatively common. The incidence increases during the peripubertal years during the period of rapid skeletal growth and is higher among boys than girls.1,2 There is evidence from several studies that low bone mass is a predictor of future fracture. Thus, in a series of girls with distal forearm fracture, bone mass was lower than a group of matched controls.3 The results of a systematic review of case-control studies and a recent prospective study suggested that there is an association between low BMD and fractures in children.4 Such fractures seem therefore to be a marker of bone fragility in childhood.

There is strong evidence to suggest that fractures in later life are linked with an increased risk of future fractures.5,6 There is some evidence that peri/premenopausal fractures are linked with an increased risk, and in one study, fractures as early as age 20 were linked with future fracture risk.7–11 There is, however, to our knowledge no good evidence linking childhood fracture to the risk of fracture in later life. Such data are important—evidence that childhood fractures are linked with an increased risk of future fracture provides an important rationale for considering affected individuals for assessment and therapy to prevent further bone loss and reduce morbidity in later life. Also identification of a history of fracture in childhood as a risk predictor of future fracture would help inform the development of more targeted risk assessment tools. We used data from the European Prospective Osteoporosis Study (EPOS) to determine whether fractures reported in childhood were linked with an increased risk of fractures in later life.

MATERIALS AND METHODS

Subjects

The subjects were recruited for participation in the European Prospective Osteoporosis Study (EPOS). The...
detailed methods concerning the baseline phase have been described elsewhere. In brief, men and women ≥50 yr of age were recruited from population registers in 36 European centers. Stratified sampling was used with the aim of recruiting equal numbers of men and women in each of six 5-yr age bands: 50–54, 55–59, 60–64, 65–69, 70–74, and ≥75 yr. Subjects were interviewed using a structured interview that included questions about previous fractures. Subjects were asked “Have you ever suffered from a broken bone (fracture)?” If yes, subjects were asked about the site of their previous fracture(s) (vertebral, hip, rib, forearm, other), number of fractures, the age of their first fracture (at each site), and the level of trauma (spontaneous, minor, or major trauma) for that fracture.

Lateral spinal radiographs were performed to ascertain prevalent vertebral deformities. The radiographs were evaluated morphometrically by one of three observers and the presence of vertebral deformity determined using the McCloskey-Kanis method. (13)

Follow-up

The subjects recruited in 29 centers were followed prospectively by annual postal questionnaire and in a further 3 centers by telephone or personal interview. However, because of a low follow-up rate, data from one center were subsequently excluded from the analysis. Subjects were asked to record details of any fractures sustained in the intervening period, including marking on a body manikin (included in a previously validated postal questionnaire) the position or site of their fractures. (14) Fractures reported were verified at each of the participating centers by the principal investigator by review of radiographs, medical record, or subject interview. From these sources, contemporary data to confirm or refute the occurrence and site of fracture were not available in 9% of cases. In these cases, the site of fracture was determined from the area marked by the subject on the manikin. (14)

BMD measurements

A subsample of 21 centers was able to measure BMD at the hip and or the spine at baseline or during follow-up in subsamples of between 20% and 100% of their available participants using DXA. The densitometers in each center were, with one exception (a Sopha fan-beam machine), pencil beam DXA machines made by Lunar, Hologic, or Norland. They were cross-calibrated using the European Spine Phantom (ESP). (15) The ESP is a semianthropomorphic phantom with three “vertebrae” of known densities 0.5, 1.0, and 1.5g/cm². (15) At least five measurements of the phantom were made on each machine, and a two-parameter empirically fitted linear or exponential calibration curve used to convert measured density values into standardized values, as previously described. (16) For the participants considered in this analysis, 4807 (36%) from 19 centers had hip BMD (femoral neck and/or trochanter) measurements, and 3998 (30%) from 14 centers had spine BMD measurements. Those who had a scan were slightly younger than those who did not (63.1 versus 63.6 yr); however, there was no difference in the proportion of

| TABLE 1. Occurrence of Recalled Childhood Fractures (Age 8–18 yr), Prevalent Vertebral Deformities, and Incident Limb Fractures in Men and Women |
|-----------------|-----------------|
|                 | Men (n = 6451)  | Women (n = 6936) |
| Prevalent vertebral deformity | 717 (11.6) | 740 (11.2) |
| Any recorded adult incident fracture* | 215 (3.3) | 472 (6.8)* |
| Any recorded adult incident limb fracture | 140 (2.2) | 391 (5.6)* |
| Any fracture† aged 8–18 yr | 574 (8.9) | 313 (4.5)* |
| Forearm fracture aged 8–18 yr | 239 (3.7) | 130 (1.9)* |

Subjects who reported a childhood fracture between the two groups.

Analysis

The analysis was restricted to subjects 50–79 yr of age at baseline because the proportion of the study cohort above the age of 80 yr was small. We defined childhood fractures as those that first occurred between the ages of 8 and 18 yr. The younger age limit was chosen pragmatically because of concerns about recall of earlier fractures. In the prospective phase incident limb fractures were classified using the ninth edition of the International Classification of Diseases. (17) Cox proportional hazards model was used to assess the predictive risk of childhood fracture on the risk of nonvertebral fractures sustained during the EPOS follow-up study. Subjects contributed follow-up time (person years) from the date of the baseline survey until limb fracture, death, or the end of the study. In subjects who sustained more than one incident fracture of the same type during follow-up, the time to the first fracture event was used in the analysis. The results of this analysis were expressed as hazards ratios (HRs) and 95% CIs.

We looked also at the association between self-reported childhood fractures and prevalent vertebral deformity (identified from morphometry at the baseline survey). For this analysis, we used logistic regression with the results expressed as ORs and 95% CIs. All analyses were undertaken separately in men and women with adjustments made for center. Analyses were performed using the statistical package STATA. (18)

RESULTS

Subjects

A total of 6451 men (mean age, 63.8 ± 8.0 [SD] yr) and 6936 women (mean age, 63.1 ± 7.9 yr) were followed for a median of 3 yr (range, 0.4–5.9 yr), for a total of 41,042 person-years of follow-up. At baseline, 717 (11.6%) men and 740 (11.2%) women had evidence of a prevalent vertebral deformity (Table 1). During the follow-up period, there were 391 incident limb fractures in women and 140 in men.
Occurrence of childhood fractures

Childhood fractures between the ages of 8 and 18 yr were reported by 574 (8.9%) men and 313 (4.5%) women. Of these, forearm fractures were the most frequent (men = 239; women = 130; Table 1). Both history of "any" and forearm fractures were more common in men than women. The mean age for occurrence of any fracture was slightly greater in men than women (13.3 versus 12.8 yr; p < 0.05). The frequency of occurrence of first reported fracture (any site) by age in both men and women is shown in Table 2. If an individual had sustained fractures at different sites (between 8 and 18 yr), the age of the earlier fracture was included.

Childhood fracture and risk of future fracture

There was no association between the occurrence of childhood fracture (any site) and future incident fracture or incident limb fracture in men and women (Table 3). Small numbers at individual fracture sites precluded analysis, for example, of the association between childhood forearm fractures and future fracture. The risk of incident fracture was not influenced by follow-up time. There was no association between the occurrence of childhood fracture and the risk of prevalent vertebral deformity (ascertained at the baseline survey) in either sex (Table 3).

Childhood fracture and BMD

In both men and women, BMD measurements at the spine and femoral neck were similar among those who did and did not report sustaining a fracture during childhood (Table 4). This was true also when analysis was restricted to those with a childhood forearm fracture.

DISCUSSION

In this prospective study, childhood fractures were not associated with an increased risk of subsequent limb or vertebral deformity.

Our study had several advantages: data concerning incident fractures were collected prospectively, it was population based, and it included both men and women. There are, however, several limitations that need to be considered when interpreting the results. Classification of childhood fractures at the baseline survey was based on self-report of events that occurred decades earlier and subject therefore to errors of recall. When compared with fracture rates in population-based studies from Scandinavia, the United Kingdom, and the United States, the reported fracture rates seem lower, although the epidemiological pattern is similar in that fractures were more common in boys than girls and occurred at an older age in boys. In the United Kingdom, data from the General Practice Research Database suggest that around one third of boys and girls sustain at least one fracture before 17 yr of age, with a peak annual incidence in boys of ~3% and in girls of 1.5%. There is evidence, however, that there has been a secular increase in the occurrence of fracture from several countries, which may in part explain the apparent difference. In Rochester, MN, fracture rates from ages 0 to 34 yr increased by 32% in male residents and 56% in female residents between 1969–1971 and 1999–2001, with most of the excess occurring in those 0–20 yr of age. In Malmo, Sweden, there was an increase in distal forearm fractures of ~60% in girls and 35% in boys between 1950 and 1979. We cannot, however, exclude under-reporting as a possible cause. In our study, we asked about age at first fracture rather than specifically about childhood fractures, and it may be that, for some participants, these fractures may not have been considered relevant or important. Recall is likely to have been poor in relation to age of fracture; however, our main analysis was based on occurrence rather than timing of fracture. We looked separately at individuals with reported childhood fractures between 8 and 14 and 15 and 18 yr on the basis that recall may have been better for the later fractures; however, the results of the analyses looking at future fracture risk were similar for the two groups. The occurrence of a recent fracture may have influenced recall of childhood fracture; however, given that the study was prospective with incident fractures occurring after the baseline assessment, this would be relevant for prevalent vertebral deformities only and would tend if anything to bias the results in favor of a positive association.

Errors may also have occurred in the classification of incident fractures. To reduce the risk of over-reporting, fractures were where possible confirmed by either review of the radiograph or contemporary medical records or subject interview. In a small proportion of cases (9%), it was not possible to confirm fracture by any of these methods; however, restricting the analyses to those individuals in whom fractures were confirmed did not affect the results (data not shown). Given the study design, it was not possible to assess the degree of under-reporting. In a separate study, however, among 174 subjects with a known history of previous fracture, only 12 (7%) did not recall the event, and only 3% of subjects did not recall a hip or distal forearm fracture. The effect of any under-reporting would tend to reduce the chance of finding any significant association between childhood fracture and future limb fracture.
deformity—we used a morphometric approach with good specificity and have shown a significant association between deformities defined using the method and low BMD.(13,21) Finally our results were derived from a predominantly white population in Europe who sustained childhood fractures over 50 yr ago, and the data should be extrapolated beyond this population with caution.

Data from both cross-sectional and prospective studies suggest that the presence of a prior fracture is a strong predictor of future fracture.(5,6) The risk seems greater for vertebral deformity predicting subsequent vertebral deformity, although there is an increased risk for any fracture.(5) The increased risk seems in part related to, although independent of, BMD.(6) In several studies, peri-/premenopausal fractures have been shown to predict the risk of future fracture.(7–11) The mechanism by which fracture increases risk of future fracture is unknown, although it is thought in part to relate to reduced bone fragility and possibly an increased risk of falls. To our knowledge, there are no data concerning the relationship between childhood fracture and future fracture risk. Our data suggest no increased risk of future fracture or prevalent vertebral deformity linked with childhood fracture. Information was available on incident vertebral deformities; however, the numbers were too small to allow any meaningful analyses.

There is evidence that fractures during adolescence are linked with a reduced bone mass.(2–4) Whereas BMC continues to accrue during growth, there is dissociation between increase in height and mineralization of the skeleton. This corresponds to the time of peak fracture occurrence in childhood. The higher risk of fracture in boys would suggest that trauma plays an important role also in determining susceptibility to these fractures. It is possible that with increasing age the skeletal envelope fills and therefore childhood bone fragility does not track into later life. This is supported by our findings showing no increased fracture risk linked with childhood fracture and, in the subsample with measurements, no association with BMD.

What are the implications of our findings? Our data relate to fractures that occurred many years ago and may not necessarily be relevant to current childhood fractures. Further cross-sectional and prospective studies are required looking at the predictive risk of childhood fractures, which have occurred more recently and fractures in late life. Until such results are available, it would seem prudent to optimize lifestyle factors relating to skeletal growth among children who sustain childhood fractures. In assessment of fracture risk among older men and women, however, our data would suggest that a recalled history of fracture during childhood is not an important determinant of fracture risk.

In conclusion, this study showed that childhood fractures do not seem to be linked with a significant increase risk of future fracture. In assessment of future fracture risk, a history of childhood fracture does not seem to be important.

**ACKNOWLEDGMENTS**

The study was financially supported by a European Union Concerted Action Grant under Biomed-1 (BMH1CT920182), and also EU Grants C1PDCT925102, ERBC1PDCT 930105, and 940229. The central coordination was also supported by the UK Arthritis Research Campaign, the Medical Research Council (G9321536), and the International Osteoporosis Foundation. We thank the
following individuals—Aberdeen, UK: Rita Smith; Cambridge & Harrow, UK: Anna Martin, Judith Walton; Truro, UK: Joanna Parsons; Oviedo, Spain: Manuel Naves Diaz, J. Bernardino Diaz Lopez, Ana Rodriguez Rebollar; Madrid, Spain: M. Diaz Curiel, J. Ortega.

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Received in original form March 3, 2008; revised form June 17, 2008; accepted February 11, 2009.

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