DISSERTATIONES MEDICINAE UNIVERSITATIS TARTUENSIS
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MART KULL

Impact of vitamin D and hypolactasia on bone mineral density: a population-based study in Estonia

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In memory of my father
Mart Kull (1956–2008)

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I. ABBREVIATIONS AND DEFINITIONS

BMD	bone mineral density
CaSR	calcium-sensing receptor
CI	confidence interval
DXA	dual energy X-ray absorptiometry
FGF23	fibroblast growth factor 23
HL	hypolactasia
HR-MRT	high-resolution magnetic resonance tomography
HR-pQCT	high-resolution peripheral quantitative computer tomography
IDDM	insulin-dependent (type 1) diabetes mellitus
IGF-1	insulin-like growth factor type 1
IL	interleukin family of cytokines
IOF	International Osteoporosis Foundation
ISCD	International Society of Clinical Densitometry
INF-γ	interferon gamma
LCT	lactase protein encoding gene
NHANES	National health and nutrition examination survey
NOF	National Osteoporosis Foundation
PBM	peak bone mass
PTH	parathyroid hormone
ROI	region of interest
Sv	Sievert, the SI-derived unit of radiation dose equivalent
TGF-α	tumour growth factor alpha
T_{H1}	T-helper cell subpopulation, IFN-γ elaborating
T_{H2}	T-helper cell subpopulation, IL-4, IL-5, and IL-13-producing, B-cell activation capable
TNF-α	tumour necrosis factor alpha
T_{Reg}	regulatory subpopulation of T-cells
UVB	ultra violet radiation of wavelength B
VDR	vitamin D receptor
VDRE	vitamin D response element
WHO	World Health Organization
Wnt	class of genes (originally called “wingless”) encoding several signalling molecules responsible for diverse growth and development functions in a variety of organisms, which include regulation of bone metabolism.

2. LIST OF PUBLICATIONS

Paper I: Kull M, Kallikorm R, Lember M. Bone mineral density reference range in Estonia: a comparison with the standard database (NHANES III). Journal of Clinical Densitometry. 2009; 12: 468–74.

Paper II: Kull M, Jr., Kallikorm R, Tamm A, Lember M. Seasonal variance of 25-(OH) vitamin D in the general population of Estonia, a Northern European country. BMC Public Health 2009; 9: 22.

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Paper IV: Kull M, Kallikorm R, Lember M. Body mass index determines sunbathing habits: implications on vitamin D levels. Internal Medicine Journal 2009; 39: 256–8.

Paper V: Kull M, Kallikorm R, Lember M. Impact of molecularly defined hypolactasia, self-perceived milk intolerance and milk consumption on bone mineral density in a population sample in Northern Europe. Scandinavian Journal of Gastroenterology 2009; 44: 415–21.

Personal contribution

Mart Kull was involved in study planning, protocol conception, and subject recruitment for all the papers. Participated in questionnaire data and serum sample obtainment, bone mineral density measurements with analysis and extraction of the clinical cohort data from the densitometry database for all papers. The author also performed all the study material statistical analyses and writing of the final papers.

3. INTRODUCTION

Osteoporosis is major public health problem around the world due to the increased morbidity and mortality and decrements in the quality of life in those affected by osteoporotic fractures. The projected increase in life expectancy is going to further increase the burden of this disease.

Osteoporosis is a multi-factorial disease – several factors contribute to the decrease in bone mineral density and falling tendency, which when combined eventually result in an osteoporotic fracture. These include genetic and non-genetic factors alike. It is clear that in different parts of the world due to cultural, ethnic and environmental diversity different factors may prevail in the disease process. Estonia is situated in Northern Europe at a latitude of 59° N, resulting in limited availability of sunlight in the winter season. A long tradition of dairy cattle farming and an above average level of dairy product consumption is paradoxically accompanied by a high prevalence of lactose malabsorption in the region. We also lack dairy fortification policies implemented in other northern European countries. We believe these factors make the Estonian population distinct from most other countries in the area.

The aforementioned factors and the globally increasing prevalence of osteoporosis indicate the need for studies advancing our knowledge about the pathophysiology, diagnosis, prevention and treatment of this disease in Estonia.

4. REVIEW OF THE LITERATURE

4.1. Osteoporosis and low bone mineral density

The term "osteoporosis" was introduced during the last century. The disease is characterised by low bone mineral density and deterioration of the bone micro-architecture, which compromises the strength of the bones and leads to an increased risk of fractures (Kanis et al 1994). It is often called "the silent epidemic" and constitutes a major public health problem around the world due to the increased morbidity and mortality and decrements in the quality of life in those affected by these fragility fractures (Miller 1978, Nydegger et al 1991, Chrischilles et al 1991). It has been estimated that at the age of 50 years a woman has an approximately 50% chance of sustaining a fragility fracture (Chrischilles et al 1991). The burden of fractures is predicted to grow three-fold in the next four decades due to the aging population and changing lifestyle (Cooper et al 1992, Chevalley et al 2007).

Of all osteoporotic fractures, the hip fracture contributes most to osteoporosis-related morbidity, disability and overall cost burden. Only half of those fracturing their hip return to the pre-fracture outpatient status and less than 20% have a full restoration of functioning (Miller 1978).

4.1.1. Pathogenesis and risk factors for low bone mineral density and osteoporosis

Osteoporosis is classified into primary and secondary forms based on the presence or absence of a known underlying disease or condition. Primary osteoporosis has historically been divided into postmenopausal, senile and idiopathic (incl. idiopathic juvenile) osteoporosis. A possible array of factors related to primary osteoporosis and a higher fracture risk is shown in Figure 1.

Inheritance has been suggested as playing a major role in determining bone phenotype and several genetic loci that are associated with bone mineral density have been identified (Styrkarsdottir et al 2008). However, the contribution of each such gene or polymorphism is minute, usually not exceeding a few percentage points. In addition, genetic factors are present that influence bone strength and fracture risk independent of bone mineral density (BMD) – genes regulating the macro architecture of bones and neuromuscular functioning contribute to fall propensity, which complicates finding genes that contribute to bone mineral density and peak bone mass (Ioannidis et al 2004, Ralston 2007).

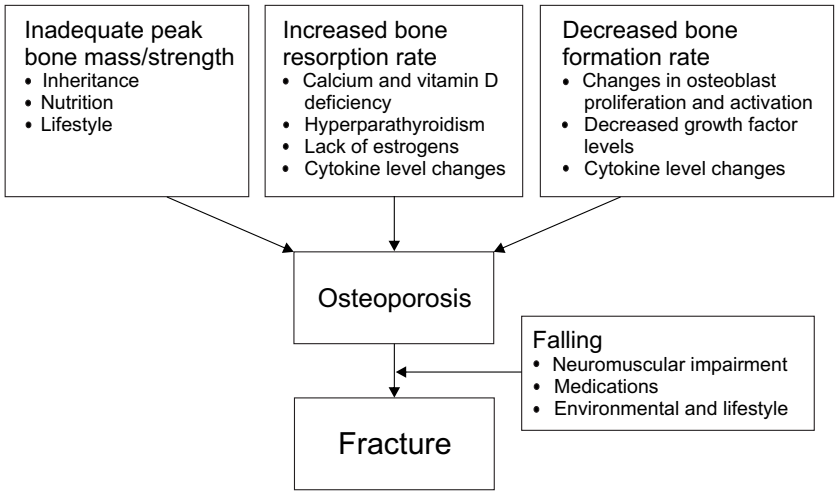

Figure 1. The factors contributing to low bone mineral density leading to fracture: several mechanisms have been identified which can in isolation or combination lower BMD and ultimately increase the susceptibility to fragility fractures.

Lifestyle and environmental factors have a significant effect on the accrual of bone mineral density (Adami et al 2003, Bischoff-Ferrari HA et al 2004). The role of vitamin D, physical activity and calcium, immobilisation, smoking and excessive alcohol intake on BMD have been established (Adami et al 2003). Overall nutritional status, protein intake, micronutrients (vitamins B6, B12, and folic acid) and their interactions with the genetic environment are of great scientific interest today. In addition to lowering bone mineral density, several factors also influence bone quality (van Meurs et al 2004, Tang et al 2007).

Oestrogen deficiency significantly increases bone resorption. Although mainly associated with menopause in women, studies have demonstrated an equally important role in men (Falahati-Nini et al 2000). The mechanisms through which the lack of oestrogens mediate bone loss are complicated, involving osteoblast-osteoclast interaction, cytokine expression by lymphocytes and increased oxidative stress (Ross 2003, Syed et al 2005, Almeida et al 2007).

Reduced rate of bone formation is an important contributor to skeletal fragility. It is the leading mechanism underlying glucocorticoid-induced osteoporosis and also a contributor to the normal age-related loss of bone mass (Canalis 2003). Possible mediators include, but are not limited to, oestrogen and androgens, IGF-1, TNF-α and the regulators of the Wnt signalling pathway but in part could also be related to the lack of mechanical loading (physical activity) associated with aging (Fujita et al 1990, Rosen et al 1998, Armstrong et al 2007).

4.1.2. Methods for measuring bone mineral density (BMD)

The term "osteoporosis" implies that the main characterisation of this disease is "porous bones". However, histological examinations of the bone are seldom carried out to diagnose the disease because of the risks and discomfort associated with the procedure. In 1994 the World Health Organization (WHO) Consensus Development Panel defined osteoporosis on the basis of bone mineral density and history of previous fragility fracture (WHO Study Group et al 1994b). Several technologies are available to measure bone mineral density:

Single energy photon and X-ray absorptiometry are restricted to peripheral skeletal sites, usually the forearm. The machines are portable and relatively inexpensive and, like dual energy X-ray absorptiometry, have high reproducibility and expose the subject to very low doses of radiation (Lawrenson et al 2006).

Quantitative computed tomography (QCT) enables differential measurement of cortical and trabecular bone in the spine or peripheral skeleton, but the equipment required is expensive and the radiation doses high without significant benefits in precision (Griffith et al 2008).

Broadband ultrasonic velocity and attenuation of the calcaneus, tibia, or patella have also been extensively studied. It is radiation-free and the devices are portable and relatively cheap. Recommended as a screening device, diagnosing osteoporosis based on ultrasound measures is not recommended (Damilakis et al 2007).

Lately technology in the form of high-resolution peripheral quantitative computed tomography (HR-pQCT) and high-resolution magnetic resonance imaging (HR-MRI) is becoming available, which have resolutions better than 100 μm. In addition to bone mineral density these allow us to non-invasively assess several aspects of bone micro-architecture (Grampp et al 1995). However, these methods are currently restricted to peripheral skeletal sites only, their high cost and low accessibility further limiting clinical usability (Boutroy et al 2005).

Dual energy X-ray absorptiometry (DXA) is widely used and preferred due to its ability to assess bone mass both at axial and appendicular sites, its high reproducibility, and the low doses of radiation associated with measurement (Johnston, Jr. et al 1991, WHO Study Group et al 1994b).

4.1.3. Dual energy X-ray absorptiometry (DXA) and bone mineral density

DXA-based densitometers were introduced in the 1980s. In Estonia the technology has been available since 1997 and currently the technique is easily accessible in Estonia (approximately 1 DXA machine per 140,000 inhabitants).

These scanners use two different X-ray wavelengths with different tissue absorption characteristics in bone and soft tissue, which makes precise mea-

surement of the amount of bone in the scanning field possible (Blake et al 1997). Original scanners used a pencil-beam with a single detector, resulting in scanning times extending 10 minutes or more, depending on the skeletal region scanned and the weight of the patient. Current devices utilise fan beams with high-resolution array detectors lowering the speed of scanning to usually less than a minute without increasing the radiation dose. Due to good collimation the radiation doses of the DXA technique are extremely low, being comparable to the doses of background radiation received every day (~7 μSv/day) (Blake et al 1997).

With the DXA method we can measure BMD in the clinically relevant skeletal sites (the lumbar spine and the proximal femur; Figure 2), but several other sites can also be measured (distal radius, total body, calcaneus, hand, etc.).

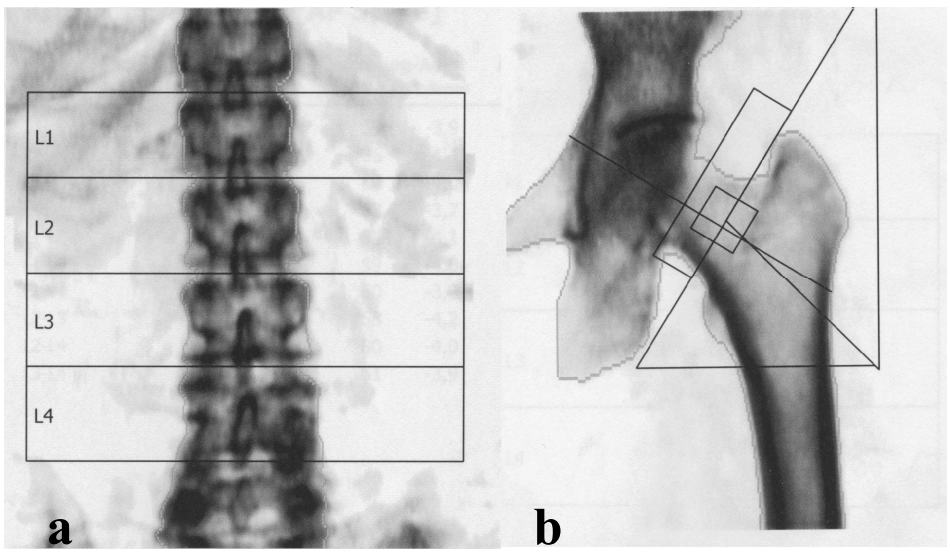

Figure 2. (a) DXA of lumbar spine (L1–L4). The mean BMD of L1–L4 vertebrae are used for diagnosis. (b) DXA of the proximal hip; BMD is measured in a number of predefined sub-regions (total proximal hip, the femoral neck, the Ward's area and the trochanter)

The total body scan supplements bone mineral density data with several anthropometric indices like fat mass, fat percentage, lean mass and total calcium content and is considered a good method for body composition assessment (Lukaski 1993, Fogelholm et al 1997). DXA-derived X-ray attenuation measurements are converted into bone mineral content (BMC; g) and the bone area is measured by calculating the projected area under the bone (BA; cm^2). From these two measurements bone density is calculated by dividing BMC by BA (BMC/BA) and expressed as areal bone mineral density (aBMD: g/cm^2).

Differences in X-ray wavelengths and bone edge detection algorithms cause variations in the resulting aBMD between different manufacturers (Genant et al 1994). For the ease of interpretation and also aiming to reduce these inter-manufacturer differences, the makers of DXA machines have implemented a derived measurement of bone density called the T-score, which is calculated using the peak bone mass (PBM) of a young reference population and is expressed as a difference in standard deviations (SD) from the mean of young healthy adults.

$$T-score = \frac{\left(SubjectsBMD / YoungAdultsBMD\right)}{YoungAdultsSD}$$

The WHO originally defined osteoporosis as a lumbar spine (L1–L4) or femur neck T-score of -2.5 SD below the mean of a young healthy population (WHO Study Group et al 1994b). The International Osteoporosis Foundation (IOF) and the International Society of Clinical Densitometry (ISCD) have recommended that the femur neck T-score be preferably used for diagnosis (Baim et al 2008, Kanis et al 2008). Such recommendations are based on large prospective studies which have demonstrated that using multiple regions and diagnosing by the lowest T-score does not improve fracture prediction when measured as a gradient of risk per standard deviation change (Kanis et al 2005, Kanis et al 2006). For children and younger individuals an age-matched analogy of the T-score, called the Z-score, is used.

$$Z-score = \frac{\left(SubjectsBMD / AgeSexMatchedBMD\right)}{AgeSexMatchedSD}$$

The comparison is made with the mean bone mineral density of sex and age-matched individuals. A Z-score of less than 2.0 SD is considered a low bone mass for specific age.

4.1.4. The impact of bone mineral density reference population in osteoporosis diagnosis

The selected reference population (geographical location, ethnicity, sampling method) have been shown to influence the DXA T-score and the resulting diagnostic decision (Melton 1997, Ahmed et al 1997). T-score has been shown to depend on several factors not related to the bone strength of the measured individual: in addition to the manufacturer and the model of the DXA machine, differences in T-score calculation techniques and factors such as the homogeneity and size of the reference population as well as differences in variability between the anatomical regions measured influence SD and hence the T-score (Greenspan et al 1996, Faulkner et al 1999). With the same mean BMD but a

wider SD margin we would identify fewer people as having osteoporosis and vice versa. Therefore, differences in reference population selection influence the dichotomisation between normal and osteoporotic individuals (Ahmed et al 1997). The US NHANES III database (published in 1995 and updated in 1998) is currently the largest reference database now including the BMD data for more than 14,000 individuals of different races and ages in the US (Looker et al 1995, Looker et al 1998). It is recommended by the IOF and the ISCD that this database be used for diagnosing osteoporosis.

The rationale for diagnosing osteoporosis using the T-score cut-off of –2.5 SD was that with this approach we found osteoporosis in approximately 30% of postmenopausal women. This corresponds with the lifetime absolute risk of osteoporotic fracture for women in the US. Originally, however, the T-score criterion was meant to be implemented as a tool for epidemiologic studies rather than making individual treatment options, which this has largely evolved into (Kanis et al 1994). Differences in BMD between individuals in different countries contribute to differences in fracture risk (Lunt et al 1997) with some of the highest rates described in the northern European countries (Johnell et al 1992, O'Neill et al 1996). Therefore it is plausible that as the threshold is based on the lifetime fracture risk of individuals, a local reference database might be superior in fracture prediction, which has led to several regional normative databases for BMD in the hip region being established and implemented in clinical practice (Kroger et al 1992a, Kroger et al 1992b, Truscott et al 1993, Lofman et al 1997, Hadjidakis et al 1997, Mazess et al 1999, Maalouf et al 2000, Dougherty et al 2001, Boonen et al 2003, Cvijetic et al 2004, Goemaere et al 2007, Ribom et al 2008, Kaptoge et al 2008, Omsland et al 2009). The recently introduced WHO fracture risk assessment tool, FRAX™, uses the NHANES proximal femur database for T-score calculation; therefore, concordance with this database is also needed if the model is to be implemented (Kanis et al 2009).

To date in Estonia no country-specific reference data are available, various osteoporosis centres use different standard databases and studies on osteoporosis often do not clarify the specific databases used in diagnosing the disease (Kumm et al 2008). This indicates a lack of consensus in the description of osteoporosis in Estonia.

4.2. Vitamin D

4.2.1. Historical background of Vitamin D

Vitamin D reached the interest of the general public and researchers with the emergence of rickets – a childhood disease of inadequate bone mineralisation usually caused by low vitamin D levels. Rickets became endemic at the end of the 19th and the beginning of the 20th centuries. In 1918 it was suggested that cod liver oil was an anti-rachitic agent (Mellanby E et al 1919). Vitamin D itself was identified and isolated from cod liver oil in 1922 with its chemical structure

determined (1928–1932) by Professor A. Windaus in Germany (McCollum EV et al 1922, Windaus A et al 1932). For this he was also awarded the Nobel Prize for chemistry (1938). The discovery in 1924 that irradiated foods contained vitamin D lead to the availability of commercial vitamin D preparations (Steenbock H 1924). This led to the disease being almost eliminated from western societies. Lately, however, cases of subclinical as well as clinical rickets are re-emerging worldwide (Holick 2006). This phenomenon could be partly attributed to the widespread public campaigns during the last decades soliciting avoidance of sun exposure with regard to its association with increased risk of skin cancer. Currently we are witnessing a "second wave" of vitamin D-related research as new and interesting functions of this "Sunshine hormone" are being discovered. These include immunomodulatory, anti-atherosclerotic and anti-cancer properties of this vitamin (Watson et al 1997, Bikle 2009, Garland et al 2009).

4.2.2. Vitamin D metabolism

Vitamin D is produced in the skin from 7-dehydrocholesterol (7-DHC) after exposure to UVB radiation (290–310 nm) producing pre-D_3 (Holick et al 1974) (Figure 3). This molecule undergoes a temperature-dependent rearrangement of its structure to form vitamin D3 and is then transported (bound to the vitamin D binding protein – DBP) to the liver (Holick et al 1974). Several hepatic cytochrome P450 enzymes are capable of converting it to the pro-hormone calcidiol (25(OH) vitamin D) (Henry 1992). This is the main circulating vitamin D metabolite and as its level is mainly regulated by substrate availability, it is used as an indicator of vitamin D status. However, this pro-hormone has a very low affinity to the vitamin D receptor (VDR) and is converted into the active hormone calcitriol (1,25(OH) vitamin D) in the renal tubular epithelium (Henry 1992). The conversion of calcidiol to calcitriol is regulated by 4 factors: a) the availability of pro-hormone 25(OH) vitamin D; b) the amount of renal 1α-hydroxylase; c) the availability of cofactors for the enzyme; and d) the activity of the 24-hyrdroxylase enzyme (CYP24 hydroxylase) (Fraser 1980). The latter enzyme competes for substrate with the 1α-hydroxylase forming an inactive metabolite (24,25(OH) vitamin D) or converts the active hormone into inactive 1,24,25(OH) vitamin D (Henry 1992). The 1α-hydroxylase level is also regulated by the level of circulating parathyroid hormone (PTH) and fibroblast growth factor 23 (FGF-23) (Schiavi et al 2004).

Transport of vitamin D metabolites between the site of synthesis and the effector tissues is carried out by vitamin D binding protein (DBP) (Birn et al 2000). As stated VDR is the intracellular mediator of 1,25(OH)2D3 function. This receptor has a very high specificity and affinity to the 1,25(OH) vitamin D molecule and has homology with other nuclear receptors of steroid and thyroid hormones (Baker et al 1988). The nuclear cascade, by which the final regulation of gene expression is mediated, is intricate and only now beginning to be elucidated.

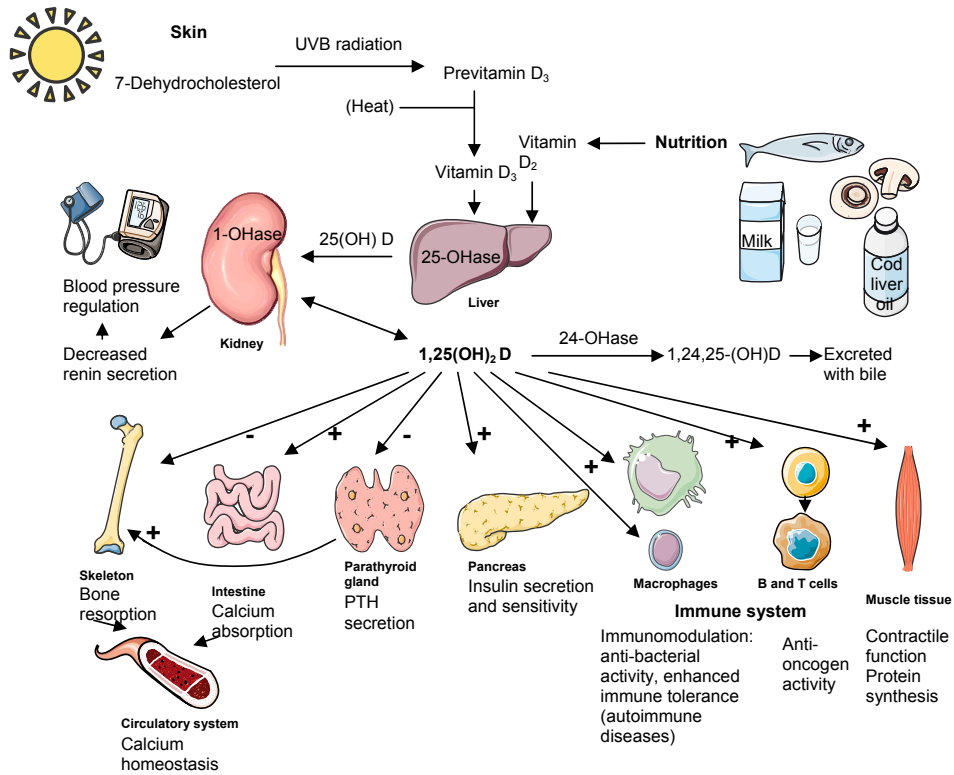

Figure 3. Vitamin D metabolism and its effect on different organ systems.

4.2.3. The role of vitamin D in bone mineral metabolism

Vitamin D is a major regulator of calcium homeostasis and bone metabolism. Vitamin D facilitates calcium absorption in the gut by increasing calcium-binding protein concentration in the small intestine (Taylor et al 1969). In addition, low levels of the vitamin lead to compensatory secondary hyper-parathyroidism aimed to retain calcium homeostasis in the presence of reduced calcium influx from the gut (Fraser 2009).

Animal studies have demonstrated that VDR null mice being fed a rescue diet rich in calcium, phosphorus and lactose prevents the elevation of PTH and the development of osteomalacia and rickets (Amling et al 1999). However, some studies support a direct effect of 1,25(OH) vitamin D on bones through the stimulation of osteogenesis (Raisz et al 1972, Yasuda et al 1998). Studies have shown that transgenic mice over-expressing VDR in osteoblastic cells have increased bone formation, which also confirms the direct effects of 1,25(OH)2D3 on bones and shows that both the formation and resorption aspects of bone metabolism are regulated by vitamin D (Gardiner et al 2000).

There is solid evidence that vitamin D has benefits with regard to fall and fracture prevention. Several longitudinal studies have demonstrated that vitamin D is an independent determinant (independent from serum and dietary calcium) of BMD. However, some, but not all, intervention studies have failed to confirm an effect of this vitamin on bone mineral density; therefore, the evidence is inconclusive (Stone et al 1998, Dennison et al 1999, Melin et al 2001, del Puente A et al 2002, Kudlacek S et al 2003, Cooper et al 2003, Bischoff-Ferrari HA et al 2004, Bischoff-Ferrari et al 2004a, Aloia et al 2005, Gerdhem et al 2005, Malavolta N et al 2005, Arabi A et al 2006, Garnero et al 2007, Hosseinpanah et al 2008, Bischoff-Ferrari et al 2009, Pasco et al 2009).Further evidence is needed to conclude if the benefits of vitamin D in the treatment of osteoporosis are solely based on better musculoskeletal functioning or if there are benefits to bone mineral density.

4.2.4. The influence of vitamin D on other organ systems and general health

Parathyroid gland. 1,25(OH) vitamin D inhibits PTH secretion but also prevents parathyroid gland proliferation. It has been suggested that it also sensitises the gland to calcium inhibition by increasing calcium-sensing receptor (CaSR) expression in this tissue (Hellman et al 2000).

Pancreas. Evidence supports the role of vitamin D in the regulation of endocrine insulin secretion. Pancreatic β-cells express VDR and calbindin-D, which modulate depolarisation-stimulated insulin release and protect against cytokine-mediated destruction of β-cells (Morrissey et al 1975, Clark et al 1980, Malaisse et al 1990, Zella et al 2003). It has been observed that vitamin D with calcium supplementation produces a significant decrease in fasting glucose and insulin resistance in patients with impaired fasting glucose (Pittas et al 2007).

Several randomised controlled trials and epidemiologic studies have shown that calcium and vitamin D supplementation decreases type II diabetes and insulin-dependent diabetes mellitus (IDDM) risk (Webb et al 1988, Pittas et al 2007, de, I et al 2008). Studies also show that vitamin D repletion and supplementation is crucial during infancy and childhood and even prenatally for the risk of developing IDDM (EURODIAB Substudy 2 Study Group 1999, Stene et al 2000, Hypponen et al 2001, Fronczak et al 2003). The reduction in IDDM risk is related to the effects of vitamin D on modulating the immune system (Dahlquist et al 1999).

Immune system. It has been demonstrated that vitamin D influences both the innate and adaptive immune system (Rook et al 1986, Penna et al 2000, Bikle 2009). Only recently has it been shown that the human cathelicidin gene has VDRE present in its promoter region (Gombart et al 2005). Its product, LL37, is a potent antimicrobial peptide (Wang et al 2004).

In the adaptive immune system 1,25(OH)D is shown to suppress proliferation and immunoglobulin production of B cells and impair the differentiation of B-

lymphocyte precursors to mature plasma cells; inhibits the proliferation of uncommitted T_H (helper) cells (Penna et al 2000), promotes differentiation of regulatory T cells (T_{REG}) and improves recruitment at the site of inflammation (Penna et al 2007).

Muscles. Both osteomalacia and its childhood analogy the rickets are clinically characterised by varying degrees of myopathy (muscle weakness). Vitamin D deficiency is the main cause of these diseases and the conditions along with the muscle symptoms respond well to treatment with vitamin D analogues. Several studies provide data on the benefit of vitamin D with regard to indices of skeletal muscle function and body sway as well as the risk of falls (Pfeifer et al 2000, Visser et al 2003, Bischoff-Ferrari et al 2004b, Bischoff-Ferrari et al 2004d). The reduction in fall propensity from improved musculoskeletal functioning is one of the anti-fracture effects associated with vitamin D in osteoporosis treatment (Bischoff-Ferrari et al 2004b, Snijder et al 2006).

Cancer. A large body of data exists documenting the inverse correlation of 25(OH)D levels with cancer incidence (John et al 1999, Ahonen et al 2000, Feskanich et al 2004, John et al 2004, Tworoger et al 2007, Abbas et al 2008). Numerous types of cancers show lower incidence/prevalence rates in populations with higher vitamin D levels. The strongest evidence is on the reduction of breast, colon, and prostate cancer incidence. The survival of cancer patients is also better in vitamin D-sufficient subjects compared with insufficient or deficient subjects (Ng et al 2008, Tretli et al 2009). These results have been confirmed in some but not all randomised controlled trials (Wactawski-Wende et al 2006, Lappe et al 2007, Chlebowski et al 2008). Several mechanisms have been proposed to be responsible for the anti-cancer effect of vitamin D and its metabolites (Garland et al 2009).

Cardiovascular system. There is evidence supporting a relation between vitamin D, blood pressure and atherosclerosis (Watson et al 1997, Vieth 1999, Willheim et al 1999, Timms et al 2002, Kasuga et al 2002). A large cohort study using the NHANES III dataset demonstrated that vitamin D levels were negatively correlated with systolic blood pressure (Scragg et al 2007). It is suggested that this effect of vitamin D is mediated both by the renin-angiotensin system and vascular smooth muscle function. (Carthy et al 1989, Li et al 2002). There is also evidence suggesting an association between low vitamin D concentrations with atherosclerosis.

In light of these diverse roles of vitamin D in the human body it is essential both for bone and general health consideration to aim for an optimal vitamin D status in any population.

4.2.6. Vitamin D status: insufficiency and deficiency

Vitamin D inadequacy is being increasingly recognised worldwide (Holick 2003, Holick 2005). This shortcoming in vitamin D is most prevalent in the

elderly, but affects people of all age groups (Chapuy et al 1996, Chapuy et al 1997, Lappe et al 2006). Vitamin D serum concentrations are influenced by several modifiable and non-modifiable factors such as diet, latitude, season, time outdoors, skin pigmentation, clothing and tanning habits (Sherman et al 1990, Budak et al 2004). It is known that with increasing latitude the availability and intensity of UVB radiation decreases. Therefore, in northern countries (above 40°N) even with adequate sun exposure dermal generation of vitamin D is absent in winter (Matsuoka et al 1988, Holick 2003). As few foods naturally contain vitamin D in considerable amounts capable of compensating this reduced vitamin D synthesis in the skin, marked seasonal variation in the levels of vitamin D has been observed in many countries (Rapuri et al 2002).

Different cut-off values for the normal threshold for 25(OH) vitamin D have been used. A level of 50 nmol/L has been widely used to define 25(OH)D insufficiency, while some studies have used 37.5 nmol/L as the lowest level of sufficiency (Malabanan et al 1998, Tangpricha et al 2002, MacFarlane et al 2004). Recent studies, however, suggest that a 25-(OH) vitamin D level as high as 75 nmol/L or higher is needed to cover all the physiological functions of vitamin D and should therefore be considered optimal (Chapuy et al 1997, Bischoff-Ferrari et al 2004c, Dawson-Hughes et al 2005, Bischoff-Ferrari et al 2006, Bischoff-Ferrari 2007). The currently recommended thresholds for vitamin D are presented in Table 1.

Estonia is situated in Northern Europe at a latitude of 59° N. Vitamin D synthesis in the skin is not possible for most of the year due to low UVB radiation intensity. The Estonian diet is scarce in foods containing vitamin D (fish and fish products) and milk products are not fortified (World Health Organization 1999). This makes Estonia a high-risk population for D-hypovitaminosis. Being vitamin D-replete is essential for a balanced calcium metabolism and healthy bones and in addition has several other benefits including better musculoskeletal functioning, reduced falls and has been associated with a lower incidence of several cancers and autoimmune diseases (Bischoff-Ferrari et al 2004a, Bischoff-Ferrari et al 2006). The seasonal variation in vitamin D levels, the prevalence of vitamin D sufficiency and deficiency and its impact on the BMD of Estonians has not previously been studied.

4.2.7. The role of sunbathing and body mass index on vitamin D

It is well known that sun-exposure (UVB wavelength radiation) is the main source of vitamin D. The radiation doses that individuals are subjected to are measured either directly using UV dosimeters or using sun-exposure questionnaires. Sunlight exposure questionnaires are commonly used to estimate UV exposure and have been shown to be reliable for various age groups and occupations (Van der Mei et al 2006, McCarty 2008).

The elderly and persons with increased body weight (fat percentage) are considered a risk group for vitamin D insufficiency (Dattani et al 1984, Arunabh et

al 2003, Parikh et al 2004). Possible explanations for this lower vitamin D level in these groups include, among others, the skin's decreased capacity to produce vitamin D and sun-deprivation (MacLaughlin et al 1985, Wortsman et al 2000). It has been questioned recently whether sunbathing habits might vary according to body mass index or total body fat percentage (Harris et al 2007). The current data are not supportive of this hypothesis that sunbathing habits are a factor explaining these lower vitamin D levels in heavier or older individuals but data are limited to elderly people only and there are no studies in wider age groups.

Table 1. Various vitamin D levels and their health implications.

Serum 25(OH) vitamin D level		Vitamin D status
(ng/mL)	(nmol/L)	
<20	<50	Deficiency
20–32	50–80	Insufficiency
32–100	80–250	Sufficiency
54–90	135–225	Normal in sunny countries
>100	>250	Excess
>150	>325	Intoxication

Reproduced from Grant WB et al (2005).

4.3. Hypolactasia and its role on milk consumption and bone mineral density

Dietary aspects are being recognised as one of the key modifiable aspects of bone health. Of these calcium intake has been the best studied, and its effect on bones has been proven in many prospective studies. Interventional and clinical trials have shown that optimal calcium intake is beneficial to bone health in almost all age groups. Dairy products are the main source of calcium for humans and hypolactasia (HL) is one key factor limiting milk intake in adults (Sahi 1978).

Hippocrates (460–370 BC) and later Galen (AD 129–200) recognised that some people experienced gastrointestinal problems after drinking milk. Only in the middle of the last century did this condition reach scientific interest (Holzel et al 1959, Durand 1960). Hypolactasia manifests by abdominal complaints after milk intake due to low lactase activity in the small intestinal mucosa, leading to malabsorption of lactose, the main carbohydrate in milk and milk products. Unabsorbed lactose is fermented by bacteria leading to symptoms (diarrhoea, flatulence, borborygmus, abdominal pain, etc.), often causing the individual to avoid non-fermented dairy products (Sahi 1978). Hypolactasia occurs as three main types: primary, secondary and congenital lactase deficiency. Although the inheritance of its most common form, primary lactase

deficiency, has been known for some time, recent DNA sequencing and haplotype analysis have provided a novel diagnostic method for diagnosing the condition. Previously methods used for diagnosing the condition (direct intestinal mucosal lactase activity measurement and lactose tolerance tests) have been either cumbersome or had inferior sensitivity and specificity (Auricchio et al 1963, Dahlqvist 1964, Soeparto et al 1972, Bond, Jr. et al 1972, Arola et al 1982, Lember 2002). A single nucleotide variant (C/T_{-13910}) in the promoter region of the LCT gene associates with the lactase activity trait in the small intestine mucosa: heterozygotes and T/T homozygotes are characterised by lactase persistence (i.e. normolactasia; NL) and C/C homozygotes by hypolactasia (Enattah et al 2002).

However, there is no complete correlation between HL and lactose intolerance (LI) (Carroccio et al 1998, Lember et al 2006). Not all subjects with HL exhibit symptoms of lactose malabsorption and vice versa. The reasons for this are that there are other factors involved in symptom acquisition: a) the composition and metabolic activities of the colonic microflora; b) the ability of the colon to remove fermentation metabolites; and c) visceral sensitivity (symptom perception) (Hammer et al 1996, Suarez et al 1997, Vesa et al 2000).

Studies suggest that HL plays a role in determining the risk of several diseases or conditions (Lember et al 1988, Meloni et al 1995, Rasinpera et al 2005).

Patients with HL and LI tend to reduce their intake of dairy products (Matlik et al 2007). The resulting lower calcium intake influences bone metabolism causing increases in bone turnover and serum PTH level leading to a decrease in bone mass (Honkanen et al 1996). Studies investigating the relationship of BMD with HL or self-reported LI in different ages and gender groups have presented contradictory results (Corazza et al 1995, Honkanen et al 1996, Kudlacek et al 2002, Obermayer-Pietsch et al 2004, Enattah et al 2005, Gugatschka et al 2007). There is evidence that HL does not have an effect on bone metabolism in populations with very low average consumption of milk products, as it is milk consumption through which the lactase trait might have its main effect on bone metabolism (Gugatschka et al 2007). The results might differ in populations with higher milk consumption. In addition the effect of HL on calcium metabolism might be modulated by vitamin D levels in countries with high frequencies of vitamin D insufficiency (Segal et al 2003).

Average milk intake differs by region, depending on several cultural and genetic factors, and Estonia with its long tradition of dairy cattle farming suggests an above-average level of dairy product consumption. The benefits of the additional calcium received from milk, its effect on bone health and the possible modulation of this effect by lactose intolerance and D-hypovitaminosis have not been studied in the region.

5. STUDY RATIONALE

Diagnosing osteoporosis using the DXA technique has been available for some time but to date no country-specific reference data on bone mineral density are available for Estonia. Throughout Estonia a variety of bone mineral density reference databases are used indicating a lack of consensus on the database to be used. Therefore, a study to determine the local reference range and a comparison of this new database with the standard (NHANES III) database is paramount in order to unify the diagnosis of this disease in Estonia.

The wide spectrum of functions in the human body makes vitamin D an essential micronutrient for bones and general health. Several climatic and nutritional factors make Estonia a high-risk population for hypovitaminosis. Studies documenting the seasonal variation in vitamin D levels or the prevalence of vitamin D insufficiency among Estonians cannot be found in the literature. Although it has been shown that vitamin D is essential for adequate calcium absorption in the body it is still not clear if this increase in calcium balance results in benefits to bone mineral density. Therefore studies further clarifying if the benefits of vitamin D in fracture prevention are solely based on better musculoskeletal functioning or if there are benefits to bone mineral density as well are missing.

It is known that sunbathing habits change with aging. However, the literature is not supportive of the hypothesis that differences in sunbathing habits could explain the lower vitamin D levels observable in heavier and older individuals. These data have been derived studying elderly people only and therefore studies in a wider age group are needed to confirm or confute these findings.

Additionally, as Estonia is historically a dairy cattle-breeding agricultural country, Estonians are suggested as having an above-average level of milk consumption (World Health Organization, 1999). This is accompanied by a high prevalence of primary hypolactasia. The benefits of the additional calcium received from milk, its effect on bone health in Estonians and the possible modulation of this effect by hypolactasia, lactose intolerance and vitamin D levels have not been studied in the region.

6. AIMS OF THE STUDY

1) To establish the normal range for bone mineral density in Estonia and evaluate its usability in diagnosing osteoporosis.
2) To investigate the vitamin D status, its determinants and seasonal dynamics in Estonia.
3) To determine if vitamin D is an independent factor determining bone mineral density.
4) To explore if body composition and body mass index influence sunbathing to an extent detrimental to vitamin D levels.
5) To analyse if bone mineral density is impacted by hypolactasia, lactose intolerance and milk consumption in Estonians.

7. MATERIALS AND METHODS

7.1. Study subjects

Population-based cohort
A random sample was drawn from the registers of two family physicians in Lääne-Viru County, Estonia. An initial invitation and a follow-up invitation (if needed) were sent to 402 randomly selected subjects to participate in the study. The selection was carried out using computer-generated random numbers in the register. Of those invited, 243 (60%) responded. The non-responders were substituted once with the next person of the same age and sex from the patient register, in order to retain the population structure of the first selection. A total of 158 substitutions were made and an invitation (and a repeat if needed) was sent to them. An additional 124 subjects responded (response rate 79%) and were included in the study. A total of 367 subjects (200 women and 167 men, aged 25–70 years) participated in the study, with an overall response rate of 66%. The final selection corresponded well with the overall population structure obtained from the national population registry (2007 census data). Study subject allocation is depicted on a flow chart in Figure 4. All study procedures and measurements in the population sample were performed between December 2005 and September 2006. The study was approved by The Ethics Committee of Tartu University and all participants signed a written informed consent form before any study specific procedures were performed.

Clinical cohort
Proximal femur bone densitometry data of 264 consecutive subjects over the age of 20 (range 21–88) attending bone densitometry in the University of Tartu Internal Medicine Department, scanned between the 1st of January 2007 and 31st of December 2007, were extracted (no personal, sensitive data were extracted from these case reports). The clinical cohort data were used to comparatively evaluate the diagnostic agreement of using the local reference range or the updated NHANES III database with regard to diagnosing osteoporosis.

7.2. Bone mineral density measurement

Of the 367 population group subjects in the study 307 agreed to a BMD measurement. All BMD measurements were done using a GE Lunar DPX-IQ densitometer (Madison, WI, US; software version 4.7e) by two IOF-certified technicians. The measured anatomical regions were the lumbar spine (L1–L4 and L2–L4), proximal femur (total femur, trochanter region and femoral neck) and total body. In all regions the results were expressed as absolute BMD (g/cm^2) and as standardised BMD (sBMD; kg/m^2). The conversion formulas used for sBMD were adopted from the papers by Lu et al (Lu et al 2001). The

WHO T-score criteria were used to distinguish osteoporosis, osteopenia (low bone mass) and normal bone mineral density (WHO Study Group et al 1994a).

The body composition indices were obtained from the total body DXA analysis. The machine quality control was performed using daily block phantom scanning and twice-weekly spine phantom scanning. The precision error for spine phantom scanning did not exceed (expressed as standard deviation) 0.010 g/cm^2 during the study. The 95% least significant change was determined for the two technologists before the study procedures in 3 regions of interest (range 0.024–0.027 g/cm^2).

7.3. General health questionnaire

All subjects in the population group completed an original questionnaire, where detailed history with current and past medication use was obtained (Appendix 1). Information regarding several aspects of lifestyle (dietary preferences, physical activity, smoking habits) as well as reproductive status and number of children and breastfed children for women were recorded. Sunbathing habits were recorded semi-quantitatively. Use of vitamin D supplements and frequency and severity of gastrointestinal complaints were recorded.

7.4. Laboratory analyses

In the population sample laboratory sampling was performed twice: from January to March and in September (2006). All samples were obtained after an overnight fast and taken between 8 AM and noon using pre-cooled serum tubes. Serum was separated and the samples stored at -20°C until analysed. The serum 25(OH)D level was measured by radioimmunosorbent assay (DiaSorin, Italy) in duplicates. The serum PTH was measured using an Immulite 2000 analyser (DPC). Vitamin D deficiency was defined as 25(OH) vitamin D level below 25 nmol/L and insufficiency below 50 nmol/L. Levels of 25(OH)D over 75 nmol/L were considered optimal.

Bone resorption marker C-telopeptide (CTX; reference range for pre-menopausal women 0.025–0.573 ng/mL and for post-menopausal women 0.104–1.008 ng/mL) and bone formation marker procollagen I amino-terminal propeptide (P1NP; reference range for pre-menopausal women 15.1–58.6 ng/mL and post-menopausal women 20.3–76.3 ng/mL) were measured using an Elecsys 2010 automatic analyser. All analyses were performed at the United Laboratory of the University of Tartu Hospital (Tartu Ülikooli Kliinikumi Ühendlabor).

The genetic analysis of the lactase (LCT) gene polymorphism was carried out at the University of Helsinki. The DNA fragment spanning the C/T-13910 variant was amplified by polymerase chain reaction (PCR) and analysed by

direct sequencing. The total volume of PCR was 50 μL containing genomic DNA (100 ng), reverse (5'-GTCACTTTGATATGATGAGAGCA-3') and forward (5'-CCTCGTTAATACCCACTGACCTA-3') primers (20 ng each), dNTPs (200 μmol/L) and 0.5 U of Taq polymerase in a standard buffer (Dynazyme, Finnzymes, Espoo, Finland). The PCR was initiated with denaturation at 95° for 10 min. (during which the enzyme was added), then 35 cycles were carried out in following conditions: denaturation at 94° for 30 s, annealing at 53° for 30 s, extension at 72° for 75 s and a final extension at 72° for 10 min. The size of PCR products was verified by 1.5% agarose gel electrophoresis with ethidium bromide.

The purification of PCR products was done by 2.5 U of shrimp alkaline phosphatase (USB) and 5 U of exonuclease I (New England Biolabs) at 37° for 60 min., after which enzymes were inactivated at 80° for 15 min. The cyclic sequencing consisted of BigDye 3.1 terminator (Applied Biosystems) according to the manufacturer's instructions with a total volume of 10 μL. Sequencing reaction was as follows: at 96° for 1 min., then 25 cycles at 96° for 10 s, at 55° for 5 s and at 60° for 4 min. To remove unincorporated nucleotides, sequencing reaction products were purified by Millipore Multiscreen plates (Millipore, US) with Sephadex G-50 superfine sepharose (Amersham Biosciences, Sweden). The sequenced products were at first electrophoresed on an ABI 3730 DNA analyser (Applied Biosystems) and then Sequencing Analysis 5.2 software (Applied Biosystems) was used for base-calling. The obtained sequence was analysed by Sequencher 4.1.4 software (Gene Codes, US).

7.5. Statistical analysis

All variables included in the analyses were verified for normality (Shapiro-Wilk test) and if skewed, an attempt to normalise the values was made using natural logarithmic transformation. Descriptive statistical methods were used to describe the demographic characteristics of the study groups. The Student t-test or the Mann–Whitney U test were used to compare continuous variables. All analyses were two-sided and a 5% probability for type I statistical errors was allowed ($p<0.05$). Statistical software R (R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria) was used in all analyses.

Paper I: The Student's t-test was used to compare baseline variables between the younger (25–39 years) and older adults (>39 years) and a t-test with summary data (assuming unequal variances) was used for comparing the national and international normative database mean BMD. The agreement in classifying into osteopenia, osteoporosis and normal individuals based upon the Estonian and NHANES III reference databases was investigated with Cohen's kappa and the Maxwell test of overall disagreement. If disagreement was present McNemar's chi-square test for matched pairs (after Liddell; 1983) was performed for the osteoporosis and osteopenia groups separately.

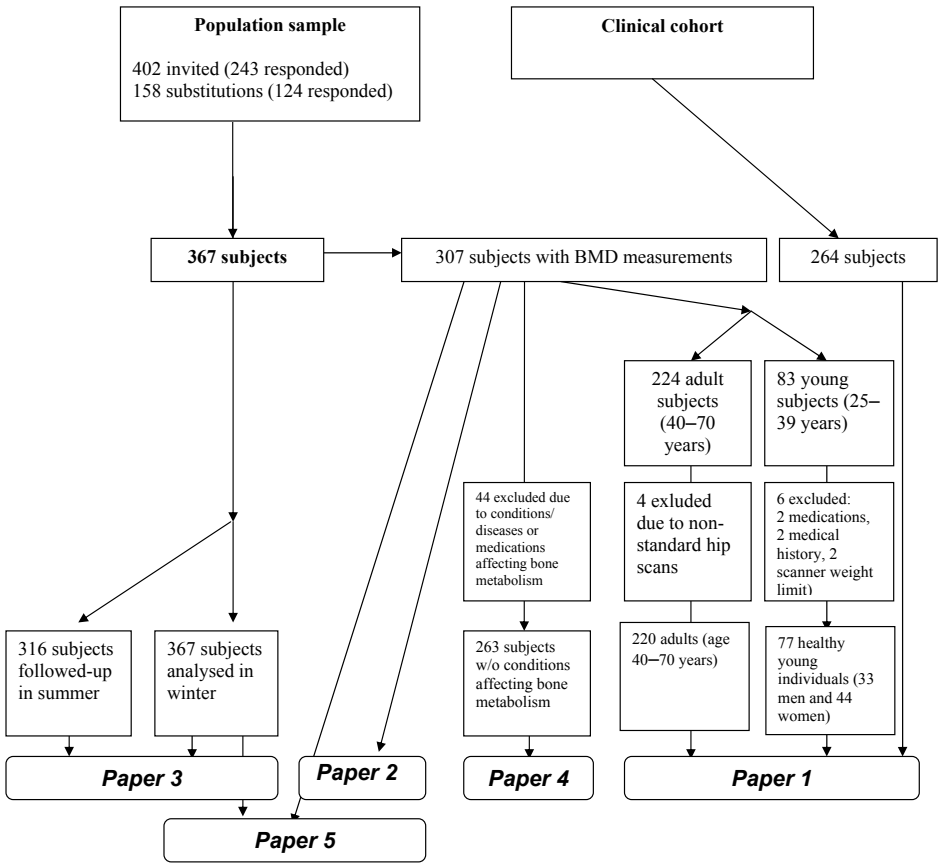

Figure 4. Study subject allocation according to different papers.

Paper II: The Pearson correlation coefficient was used to investigate the un-adjusted correlation between vitamin D and BMD. In multiple regression modelling (with BMD in various anatomical regions as the dependent variable) 25(OH) vitamin D, age, smoking (pack-years), alcohol consumption (drinks/ day), body mass index, physical activity (IPAQ score), fresh milk consumption (dL/day), caffeinated beverage consumption (cups/day), vitamin D supplement usage and total body fat percentage were used as co-variables. In addition in the women's analysis the number of children and the number of breastfed children were included.

Paper III: The Student t-test or Mann–Whitney test were used to compare means. The relationships between serum 25(OH)D concentration and PTH were studied with the nonlinear least-squares regression method for optimal vitamin D cut-off determination and analysis of variance test (ANOVA). Determinants of 25(OH)D were studied using the multiple linear regression method.

Paper IV: The chi-square test was used to determine differences in vitamin D supplement usage and sunbathing habits between quartiles of BMI or fat percentage; the Pearson correlation and multiple regression analysis was used to analyse relationships of vitamin D levels with BMI, fat percentage and age.

Paper V: The Spearman rank correlation coefficient was used to investigate the relationship between milk consumption and bone mineral density. Lumbar spine BMD and femoral neck BMD prediction models were found using the multiple linear regression method with backward selection of variables. The initial variables were molecularly-defined lactase phenotype, milk consumption, total body fat percentage, body mass index, vitamin D supplement usage, parathyroid hormone level (in winter and summer), vitamin D level (in winter and summer), smoking, coffee consumption, alcohol consumption, self-perceived milk intolerance, nationality, sex, age and occupation. All predictors with a $p<0.1$ were included in the final model. Self-perceived milk consumption and fracture probability were assessed using logistic regression.

8. RESULTS

8.1. Bone mineral density in healthy young Estonians (Paper I)

The reference values of BMD in the Estonian population were similar to the BMD values in the NHANES III corresponding age group. The mean areal BMD for the different femur sub-regions ranged from 742 to 978 g/cm^2 for women and 978 to 1064 g/cm^2 for men. The standard deviations for the mean values of BMD were similar when compared with the corresponding values in the US NHANES database (Table 2, Paper I). No significant differences between these databases were detected (p=0.06...0.9).

8.2. Diagnosing osteoporosis based on Estonian reference data (Paper I)

The T-score cut-offs for osteopenia and osteoporosis when using the female Estonian reference data were 813 and 635 for femoral neck, 624 and 447 for trochanter and 852 and 663 mg/cm^2 for total hip, respectively (Figure 5). According to the US NHANES database these numbers are 822 and 627 for femoral neck, 656 and 493 for trochanter and 833 and 649 mg/cm^2 for total hip, respectively. The resulting T-score differences ranged from -0.18 to +0.15 SDs. Implementing the local reference range into diagnosing in this clinical setting, however, resulted in some subject classification discrepancies. Additional cases of osteoporosis were diagnosed with diagnostic thresholds based on local references. Significantly more cases of osteopenia in the total hip region and fewer cases of osteopenia in the femoral neck, trochanter and combined regions were also observed when the Estonian database was used (Table III, Paper I). The apparent prevalence of osteopenia and osteoporosis was increased up to 4-fold, when combined regions instead of a single region (i.e. only femoral neck) were used in diagnosis.

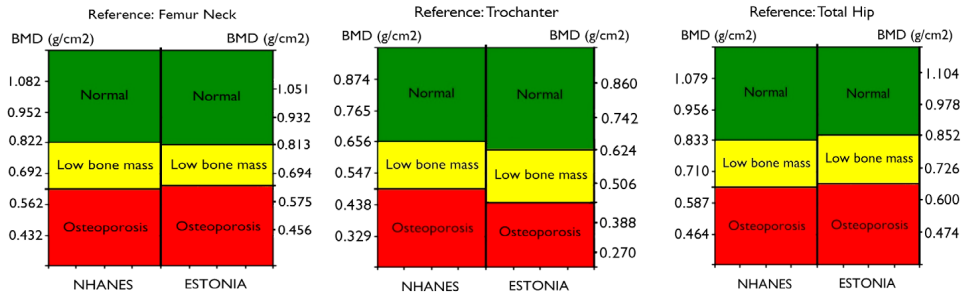

Figure 5. Comparison of Estonian and US NHANES reference data in proximal femur regions for women.

8.3 Seasonal vitamin D levels and their determinants in Estonia (Paper II)

At 44 nmol/L in winter and 59 nmol/L in summer, the mean vitamin D concentrations in Estonia during the studied seasons were well below the recommended optimal vitamin D level of 75 nmol/L. In winter more than 2/3 of the subjects (73%) were vitamin D-insufficient and at the end of summer 29% were still insufficient. Vitamin D deficiency was present in 8 percent of subjects in winter and <1% in summer. The amplitude of change between the nadir and highest levels during the year was significantly larger in men than women (13.4 nmol/L vs. 17.9 nmol/L). Based on the vitamin D/PTH response curve the optimal vitamin D cut-off for this population is around 80 nmol/L. In winter 6.4% of the subjects had elevated PTH levels.

Body mass index was in negative correlation with vitamin D levels. This, however, lost significance when the data were adjusted for sunbathing habits. It was sunbathing, smoking and vitamin D supplement usage that were significant determinants of vitamin D level in winter and sunbathing, smoking and body mass index in summer, respectively.

8.4 The independent role of vitamin D on bone mineral density (Paper III)

In unadjusted analysis, summer vitamin D levels correlated with total body and lumbar spine BMD. In the whole group in winter no correlation between BMD and vitamin D was present. In men vitamin D correlated with BMD in all the studied anatomical regions except the femoral neck. In women unadjusted analysis did not reveal any correlation between bone mineral density and

vitamin D, including subgroup analysis with only pre- or postmenopausal women.

In multiple regression analysis adjusting for age, smoking, alcohol consumption, body mass index, physical activity, fresh milk consumption, caffeinated beverage consumption, supplement usage and total body fat percentage, vitamin D level was an independent factor for lumbar spine, trochanter, total hip and total body BMD. This association is probably inherent to the more robust correlations in men (correlation not significant in the femoral neck and trochanter only), as in women the correlation after adjustment remained significant only in the lumbar spine.

8.5. Effect of body composition and age on sunbathing and vitamin D levels (Paper IV)

Analysing the self-reported sun-exposure habits recorded in the questionnaire, subjects with high body fat percentage (classification based on body fat quartiles) and overweight (BMI>30) subjects were less willing to expose their body to sunlight in summer ($p<0.0001$, ANOVA). We observed a negative correlation between BMI and vitamin D ($p=0.001$), total body fat percentage and vitamin D ($p=0.002$) as well as age and vitamin D ($p=0.04$). However, these correlations lost significance in regression analysis when sunbathing habits were introduced in the models. Vitamin D supplement usage was very low in this population sample and did not differ significantly between normal and overweight individuals.

8.6. Milk consumption, lactase persistence and bone mineral density (Paper V)

A strong positive association was observed between milk consumption and bone mineral density with considerable BMD advantages in subjects with high milk intakes ($p<0.01$). Individuals with higher milk consumption were taller and heavier without differences in BMI or body fat percentage.

Lactase non-persistence as defined by the LCT genotype resulted in lower milk consumption than in individuals with genotypes defining lactase persistence (2.0 versus 2.8...3.0 dL/day, $p=0.03$). The lactase non-persistence was, however, weakly correlated with self-perceived lactose intolerance (22% reported symptoms after ingesting fresh milk); less than half of lactose-intolerant subjects had hypolactasia and it was mainly self-perceived lactose intolerance that resulted in restriction to dietary milk consumption. Subjects with hypolactasia but milk tolerance (self-reported) did not significantly restrict their milk consumption when compared to individuals with normal lactase activity.

The lactose-intolerant subjects had lower vitamin D and higher PTH serum levels, also exhibiting higher serum bone resorption and formation markers (CTX and P1NP). This finding was more pronounced in summer. The markers of bone metabolism were not influenced by LCT genotype or lactase persistence defined by this genotype. PTH, milk consumption and age were significant determinants of lumbar spine as well as femoral neck BMD.

9. DISCUSSION

The present study is the first population-based study describing bone mineral density in Estonia. By using random sampling from the general practitioner registries in subject recruitment with a good response rate we achieved adequate overall representation of our entire population with regard to sample size (1:5000 inhabitants) and population age/gender structure. In addition to describing the normal range for BMD in Estonia we compared it with the international reference database (NHANES III) and clarified several aspects of skeletal health in Estonians.

We could not demonstrate any significant differences in the mean BMD values in any of the proximal femur sub-regions (trochanter, total hip and femoral neck) between Estonians and the NHANES population. The standard deviations were also very similar, if not smaller in our population sample despite the significantly smaller sample size. Other subject selection methods such as volunteering/advertising can cause either more health-conscious young adults with higher BMD or to the contrary individuals with known medical problems to seek knowledge about their skeletal status and participate in the study. Such self-selection has been shown to influence the reported mean BMD but can also have an expanding effect on the SD of the measured BMD (Kaptoge et al 2008). It is probable that our subject selection method explains the similarity of the SD values between our dataset and that of the US NHANES, which had a larger sample size but was partly based on volunteers. This relatively modest effect of sample size on both the convergence of mean BMD and also the SD is also demonstrated in a recent paper by Hou and colleagues (Hou et al 2008).

Although the resulting differences in the calculated T-scores were modest, when implemented into clinical decision-making we observed differences dichotomising between osteopenic and normal individuals with the two different databases. There was also apparent misclassification into osteoporosis between these databases but the differences did not reach statistical significance and should not be over-interpreted. Discrepancies in diagnosing osteoporosis with local references when compared with the standard database have also been observed by others (Hou et al 2008). Hou and colleagues showed that when using the Chinese local reference database they identified fewer subjects as having osteoporosis. This could be inherent to the genetically determined thinner bones in Asian people, which two-dimensional modalities such as DXA do not account for. Nonetheless this does once more emphasise the need for local reference databases. Our study is limited by the cross-sectional rather than prospective design and the lack of fracture data prevents us from concluding which database is superior when identifying high-fracture-risk subjects. Future prospective studies with fracture data are needed in Estonia.

In this population-based cohort of adult Estonians we demonstrated that the mean 25(OH)D in winter is well below 50 nmol/L, a level which is needed for all major vitamin D functions (Grant et al 2005). Only a third of the Estonian population reached sufficient and a negligible 3% of the population reached

optimal vitamin D status in winter. Although the summer 25(OH)D level was higher, still a third of the population did not reach sufficiency and the majority of subjects (~90%) stayed below the optimal level of 75 nmol/L. Although the winter vitamin D levels are comparable to corresponding numbers in several other European countries as shown in previous studies (Paper 3, Table 3), our summer 25(OH)D is the lowest in this comparison. This is in correlation with the reported latitude effect of vitamin D, as we are one of the northernmost countries in this comparison (Burnand et al 1992, Scharla et al 1996, Chapuy et al 1997, Carnevale et al 2001, Lamberg-Allardt et al 2001, Looker et al 2002, MacFarlane et al 2004, Ono et al 2005, Rockell et al 2006). Some caution is solicited in direct comparison of the results of these different results as not all of these studies (including our own) were part of the DEQAS vitamin D assay standardisation program.

We consider these vitamin D levels in Estonia far from adequate due to the various roles of this vitamin in the human body. To increase dietary intake of vitamin D several countries have implemented dairy and other food product fortification policies. Although the evidence on the efficacy of such fortification policies is not yet comprehensive, the present data is strongly favourable (O'Donnell et al 2008). Individuals randomised to get a fortified diet showed vitamin D levels up to 34 nmol/L higher than those on regular diets (Tangpricha et al 2003). However, there is currently no government healthcare policy in Estonia recommending dairy fortification. With high vitamin D deficiency and insufficiency prevalent throughout the year we suggest that more attention should be paid to monitoring and correcting low vitamin D levels in Estonia to assure adequate vitamin D status and guarantee optimal calcium absorption.

We also demonstrated that vitamin D is a predictor of BMD in men and this association is relatively robust as it is present in the majority of anatomical regions analysed. In women, however, the relationship is weaker, and is observable in the lumbar spine only. The few available reports on population-based samples both of men and women of a wider age group demonstrate a significant association between BMD of the hip and vitamin D status both in men and women (Bischoff-Ferrari HA et al 2004, Bischoff-Ferrari et al 2008). These studies are statistically adequately powered but recruited also volunteers, thus self-selection bias was not entirely eliminated and the studies did not adjust for factors such as fat percentage and reproductive history indicators in women. Another population-based study, however, did not find a relationship between vitamin D and BMD (except in the spine in men) (Kudlacek S et al 2003). In this study no confounders were adjusted for and vitamin D was measured only in winter. We consider these results to actually be in agreement with our data, as the correlation between wintertime vitamin D and BMD was significantly weaker in our cohort as well. The independent role of vitamin D on BMD, even though stronger in men than women, is indicative of the need to address the shortcomings in vitamin D status that are so prevalent in the Estonian population. Failure to do so might in the future further increase the burden of osteoporosis that has already become a major public health issue due to the aging population.

We were also able to demonstrate that differences in sunbathing habits between overweight and normal-weight individuals are an additional explanatory factor for the lower vitamin D levels observed in the overweight subjects. This finding contradicts existing data, which demonstrate that reduced sun exposure does not explain the inverse association of 25-hydroxyvitamin D with body fat percentage (Harris et al 2007). The existing data is on the elderly only (age 65+), whose sun-exposure is significantly lower than the average population and in our opinion could have missed the possible relationship. Our study utilised a population-based approach in a wider age group, which, in theory, provides a better assessment of this hypothesis in the general population. We cannot exclude that other factors, such as geographical location or differences in local dietary fortification policies, resulted in these contradictory results between our studies and the previous. However, even with a robust semi-quantitative approach we were able to demonstrate differences in sunbathing habits between body fat percentage quartiles and BMI subgroups, suggesting that a true negative relationship exists. We therefore conclude that at least in younger and middle-aged subjects without dietary fortification, age and body composition (fat percentage and BMI) does influence sunbathing habits to an extent detrimental to vitamin D levels.

Finally in our study we confirmed that milk consumption was an important determinant of bone mineral density in addition to anthropometric parameters (height, weight). Lactase phenotype and genotype were associated with milk consumption, but we did not observe a significant relationship between lactase genotype and phenotype with bone mineral density. Although these findings are in agreement with several previous studies (Horowitz et al 1987, Enattah et al 2004, Enattah et al 2005, Gugatschka et al 2007), deleterious effects of lactase genotype and phenotype have been reported (Honkanen et al 1996, Segal et al 2003, Obermayer-Pietsch et al 2004). Such a discrepancy between previously published studies might arise from the different diagnostic methods with different sensitivity and specificity characteristics used in these studies (Lember 2002). We complement the existing body of evidence by showing that neither LCT genotype nor phenotype defined by direct molecular analysis impacts bone mineral density in a population with high milk consumption (average ~320 mg of milk calcium/day), high vitamin D insufficiency and moderate HL prevalence.

It was the self-perceived LI that most significantly reduced milk consumption. The bone turnover markers were elevated in this group compared with lactose-tolerant subjects, suggesting the clinical significance of this finding. Not all lactose-intolerant subjects had hypolactasia when classified based on the LCT gene polymorphism analysis. As milk consumption is a significant contributor to bone health, future studies should be carried out to clarify the benefits of this molecular method for distinguishing between true adult-type hypolactasia and other conditions in which lactose ingestion is not the cause of symptoms. In the latter group diet counselling can avoid self-imposed dietary restrictions that can lead to non-optimal calcium balance and eventually lead to decrements in bone density.

10. CONCLUSIONS

1. The proximal femur bone mineral density reference range in Estonia does not deviate statistically from the standard database derived from the US NHANES III data. The mean BMD difference does not exceed 41 g/cm^2 and standard deviation difference 23 g/cm^2.
2. Using this local database for T-score calculation results in BMD classification discrepancies. Significantly more cases of osteopenia in the total hip region and fewer cases in the femoral neck ($p=0.04$), trochanter (<0.001) and combined regions ($p=0.03$) are identified using the local database.
3. A significant amplitude of vitamin D levels throughout the year in Estonia is present. In women the mean difference between the peak and nadir of vitamin D levels is 13.4 nmol/L and for men 17.9 nmol/L. The mean vitamin D level in winter is 43.7 ± 15.0 nmol/L resulting in a large proportion of the population being vitamin D-insufficient (73%) and a high prevalence of vitamin D deficiency (8%) during the winter season. As the mean summer 25(OH) vitamin D level is 59.3 ± 18.0 nmol/L only a small proportion of the population has optimal vitamin D levels even at the peak vitamin D season.
4. BMD in the highest quartile of summer 25(OH) vitamin D is 4.2% to 10.2% (P values 0.1...0.001) higher than in the lowest summer vitamin D quartile. Vitamin D is an independent factor for lumbar spine, trochanter, total hip and total body bone mineral density. However, the association is stronger in men than in women.
5. Both body mass index and body fat percentage are significantly associated with sunbathing habits and result in lower levels of vitamin D in overweight subjects.
6. Regular milk consumption is a significant determinant of bone mineral density in this population with high vitamin D insufficiency and high hypolactasia. Regular milk consumption results in significant benefits to BMD (5.1–6.5%; $p=0.03$–0.05).
7. Milk consumption is impacted by self-perceived LI rather than hypolactasia. Lactose-intolerant subjects exhibit an increase in bone metabolism markers CTX ($p=0.01$) and P1NP ($p=0.085$) when compared with lactose-tolerant subjects.
8. The LCT gene variant 13910 associated with the lactase activity trait in the body does not influence bone mineral density in this population-based cohort.

11. SUMMARY IN ESTONIAN

D-vitamiini ja hüpolaktaasia mõju luu mineraalsele tihedusele: rahvastikupõhine uuring Eestis

11.1. Uuringu eesmärgid

1) Kirjeldada luu mineraalse tiheduse normiväärtusi Eestis ja hinnata kohaliku normandmebaasi kasutatavust osteoporoosi diagnostikas.
2) Leida D-vitamiini vaeguse esinemissagedus, sesoonne varieeruvus ning seda mõjutavad tegurid Eestis.
3) Kontrollida, kas D-vitamiin on oluline ja sõltumatu luu mineraalset tihedust mõjutav tegur Eestis.
4) Hinnata, kas keha rasvaprotsent ja kehamassiindeks mõjutavad inimeste päevitusharjumusi määral, mis mõjutab D-vitamiini taset.
5) Analüüsida hüpolaktaasia, rõõsa piima talumatuse ja piima tarbimise mõju eestlaste luu mineraalsele tihedusele.

11.2. Uuritavad ja meetodid

Uuritavad valiti juhuslikkuse printsiibil Lääne-Virumaa perearstide nimistutest. Kutsutud 560 inimesest osales uuringus 367 uuritavat (200 naist, 167 meest, vanuses 25–70 aastat, vastanute protsent 66%) ja lõplik uuringugrupi vanuseline ja sooline struktuur oli kooskõlas Eesti rahvastikuregistris registreerituga.

Eesti normiväärtuste ja standardse NHANES III andmebaasi ühtivuse hindamiseks osteoporoosi diagnoosimisel kasutati 264 ambulatoorselt Tartu Ülikooli osteoporoosi kabinetti pöördunu (uuringud teostatud 01.01.2007–31.12.2007) luu mineraalse tiheduse andmeid.

Rahvastikurühmast (367 uuritavat) nõustus luu tiheduse määramisega 307 uuritavat. Luu mineraalse tiheduse määramiseks kasutati GE Lunar DPX-IQ densitomeetrit. Mõõdeti lülisamba lumbaalosa, proksimaalse reieluu ning kogu keha keskmine luu mineraalne tihedus, mis analüüsimiseks teisendati standardiseeritud ühikutele (sBMD; g/cm^2). Keha rasva protsent määrati DXA-ga kogu keha kompositsiooni hindamise meetodil.

Küsimustikuga selgitati kaasuvad haigused, kasutatavad ravimid, reproduktiiv anamnees, päikesevõtmise harjumused (pool-kvantitatiivselt) ning rõõsa piima tarbimisest tingitud kaebuste esinemine.

Kahel korral aastas määrati uuritavatel vereseerumi 25(OH) D-vitamiini (Diasorin) ja parathormooni tase (DPC) ning talvel luu ainevahetuse markerid CTX (seerumi C-telopeptiid, Elecsys) ja P1NP (prokollageen1 N-terminaalne propeptiid, Elecsys) tase. Laktaasi geeni 13910 C/T polümorfism 2. kromosoomi pikal õlal (2q21.3) selgitati PCR amplifikatsioonile järgnenud sekveneerimise alusel (Sequencher, Gene Codes).

Statistilises analüüsis kasutati vabavaralist tarkvarapaketti R (R Foundation for Statistical Computing). Demograafiliste andmete esitamisel kasutati kirjeldavaid statistikameetodeid. Studenti t testi ning Mann-Whitney U testiga võrreldi pidevaid tunnuseid. Lubatud statistilise vea piiriks valiti 5% ($p<0.05$). Teised kasutatud statistilised meetodid: Maxwelli test, hii-ruut test, McNemari hii-ruut test (Liddelli järgi; 1983), Pearsoni korrelatsiooni koefitsent, Spearmani astmeline korrelatsiooni koefitsent, mitmene regressiooni analüüs, mitte-lineaarne regressiooni analüüs, logistiline regression ja variatsiooni analüüs (ANOVA).

Uuringul on Tartu Ülikooli Eetikakomitee luba.

11.3. Tulemused

Eestlaste luu mineraalne tihedus reieluukaela piirkonnas.
Eestlaste luu mineraalse tiheduse normiväärtused on sarnased NHANES III andmebaasis avaldatud väärtustega. Erinevate anatoomiliste piirkondade luu mineraalse tiheduse keskmised väärtused ja nende standardhälbed on kahes omavahel võrreldud andmebaasis sarnased (statistiliselt olulisi erinevusi ei tuvastatud; $p>0.05$). Nimetatud andmebaaside rakendamisel osteoporoosi ja osteopeenia diagnoosimisel esinevad lahknevused. Kasutades Eesti andmebaasi suureneb mõnevõrra osteoporoosi esinemissagedus ja leiame oluliselt rohkem osteopeenia juhte proksimaalse reieluu osas. Samas leiame vähem osteopeenia juhte kui kasutame diagnoosimiseks reieluukaela, reieluu trochanter'i või kombineeritud anatoomilisi piirkondasid.

D-vitamiini sesoonne kõikumine ja seda mõjutavad tegurid.
Keskmine talvine D-vitamiini tase eesti täiskasvanud elanikkonna seas oli 44 nmol/L ja suvine 59 nmol/L. Talvel 2/3 (73%) ja suve lõpus ~1/3 (29%) kannatasid D-hüpovitaminoosi all. Raske D-vitamiini puudus e. defitsiit esines talvel 8%-l ja suve lõpus 1% uuritutest. Talvel oli paratüreoidhormooni tase tõusnud 6.4%-l uuritavatest. Peamisteks D-vitamiini taset määravateks teguriteks regressioonianalüüsi alusel olid päevitamine, suitsetamine, kehamassiindeks ja D-vitamiini sisaldavate toidulisandite tarvitamine.

D-vitamiini iseseisev mõju luu mineraalsele tihedusele.
Kaasuvatele mõjuritele kohandamata analüüsis korreleerus D-vitamiini tase nii kogu keha kui lülisamba luu mineraalsele tihedusega. Talvise D-vitamiini tase ei korreleerunud luu mineraalse tihedusega. Seos luu mineraalse tiheduse ja D-vitamiini vahel oli tugevam meeste hulgas. Regressioonimudelis, kohandades analüüsi vanusele, suitsetamisele, alkoholi kasutamisele, kehamassi indeksile, füüsilisele aktiivsusele, piima ja kohvitoodete tarbimisele, D-vitamiini sisaldavate toidulisandite tarbimisele ja keha rasva protsendile, oli D-vitamiin oluliseks luu mineraalset tihedust mõjutavaks teguriks kogu keha, lülisambas ja reieluu trochanter'i piirkonnas.

Keha rasva protsendi ja kehamassi indeksi mõju päevitamisharjumustele.
Võrreldes madala või normipärase kehamassiindeksiga ja/või rasvaprotsendiga isikutega päevitasid ülekaalulised (KMI>30) ja kõrge keha rasvaprotsendiga uuritavad oluliselt vähem (ANOVA $p<0.0001$). Negatiivselt korreleerusid omavahel D-vitamiin ning kehamassi indeks ($p<0.001$), D-vitamiin ning keha rasvaprotsent ($p=0.002$) ja D-vitamiin ning vanus. Analüüsi kohandamine päevitamisharjumustele vähendas nende seoste tugevust alla seatud statistilise olulisuse nivood ($p>0.05$). Keha rasva protsent ja kehamassi indeks mõjutavad oluliselt eestlaste päevitamisharjumusi. Käesolevas analüüsis ei olnud keha rasva protsent, kehamassindeks ega vanus iseseisvateks D-vitamiini mõjutavateks teguriteks. D-vitamiini sisaldavate toidulisandite tarbimine oli eestlaste hulgas väga harv (~3%).

Piima tarbimise ja hüpolaktaasia seos luu mineraalse tihedusega.
Piima tarbimine on oluliseks luu mineraalset tihedust mõjutavaks teguriks eestlastel. Geenianalüüsiga määratletud hüpolaktaasia olemasolu tingis võrreldes normolaktaasiaga isikutega väiksema rõõsa piima tarbimise (2.0 versus 2.8...3.0 dL/p, $p=0.03$). Hüpolaktaasia ja rõõsa piima talumatus korreleerusid omavahel nõrgalt (22% HL isikutest tundsid sümptoome peale rõõsa piima tarbimist ja vähem kui ppooltel rõõska piima mitte taluvatest isikutest esines ensüümi laktaas vähesus). Peamiseks piimatarbimist piiravaks teguriks osutus rõõsa piima talumatus, mitte hüpolaktaasia. Uuritavad, kellel oli diagnoositud hüpolaktaasia, kuid kes subjektiivselt talusid rõõska piima tarvitasid piimatooteid sama palju kui normolaktaasiaga isikud ($p>0.05$).

Rõõska piima mitte taluvatel isikutel oli oluliselt madalam D-vitamiini tase ning kõrgem paratüreoidhormooni tase, ning tõusnud luu ainevahetuse markerite (CTX ja P1NP) tase vereseerumis. Luumarkerite taset ei mõjutanud LCT genotüüp ega hüpolaktaasia olemasolu.

II.4. Järeldused

1. Reieluu mineraalse tiheduse normiväärtused Eesti täiskasvanud rahvastiku hulgas ei eristu oluliselt NHANES III standardandmebaasis esitatutest. Maksimaalne erinevus keskmises luu mineraalses tiheduses oli 41 g/cm^2 ja standardhälbe osas 23 g/cm^2.
2. Lahknevused esinevad Eesti andmebaasi rakendamisel osteoporoosi ja osteopeenia diagnoosimises – kohalikku andmebaasiga leiame oluliselt rohkem osteopeenia juhte kogu proksimaalse reieluu piirkonna ($p=0.04$) ning vähem osteopeenia juhte reieluukaela ($p=0.04$), trochanter'i ($p<0.001$) ning kõigi reieluu piirkondade kobineeritud luutiheduste alusel diagnoosides ($p=0.03$).
3. Erinevate aastaaegade lõikes oli D-vitamiini taseme kõikumine Eestis suur. Naistel oli minimaalse ja maksimaalse D-vitamiini taseme vahe keskmiselt 13.4 nmol/L ja meestel 17.9 nmol/L. Keskmine D-vitamiini tase talvel oli

43.7 ± 15.0 nmol/L, mistõttu suurel osal uuritutest oli talve madal (73%) või väga madal (8%) D-vitamiini tase. Keskmine D-vitamiini tase suvel oli 59.3 ± 18.0 nmol/L mistõttu oli optimaalne D-vitamiini tase ka suvisel perioodil väga vähestel.

4. Luu mineraaleinetihedus suvise D-vitamiini kõrgeimas kvartiili kuuluvatel isikutel oli 4.2–10.2% (p=0.1–0.001) kõrgem kui madalaimasse kvartiili kuuluvatel isikutel. D-vitamiini tase oli iseseisvaks luu mineraalse tiheduse mõjuriks lülisamba, trochanter'i, proksimaalse reieluu ning kogu keha anatoomilistes piirkondades. See leitud seos on meestel tugevam kui naistel.
5. Kehamassiindeks ning keha rasvaprotsent mõjutavad eestlaste päevitamisharjumusi ja olid üheks põhjuseks, miks tüsedatel inimestel on madalamad D-vitamiini väärtused. Uuritavad, kes väldivad päevitamist on oluliselt kõrgem KMI (30.3 vs. 26.9, p=0.006) ja kõrgeimasse ning madalaimasse keha rasva protsendi kvartiili kuuluvad isikud erinesid teineteisest oluliselt oma päevitusharjumuste poolest.
6. Rõõsa piima tarbimine on oluline luutihedust määrav tegur kõrge D-vitamiini vähesuse ja hüpolaktaasia levimusega rahvastikus. Regulaarne piima tarbimine tagab oluliselt kõrgema luu mineraalse tiheduse (5.1–6.5%; p=0.03–0.05).
7. Pigem tunnetuslik rõõsa piima talumatus kui hüpolaktaasia mõjutab piimatoodete tarbimist. See avaldab mõju luude ainevahetusele: piima mittetaluvatel isikutel oli oluliselt kõrgem luumarkerite CTX (p=0.01) ja P1NP (p=0.085) tase.
8. Laktaasi geeni (LCT_{13910}) polümorfism mis määrab laktaasi aktiivsuse soolestikus, ei avalda iseseisvat mõju luu mineraalsele tihedusele uuritud rahvastikurühmas.

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APPENDIX I

Väike-Maarja elanike tervise uuring Kuupäev: ___/___/200__.

Üldine

1. Mis rahvusest Te olete _____

	Rahvus
Isa	
Isapoolne vanaisa	
Isapoolne vanaema	

	Rahvus
Ema	
Emapoolne vanaisa	
Emapoolne vanaema	

2. Millega tegelete?

Õpin	**Töötan**	**Ei tööta**
	Kontoris	Vanaduspensionär
	Füüsilisel tööl	Haiguse tõttu
	Teen mõlemat	Tööd ei ole

Teie haigused

3. Kas olete põdenud või põete mõnda kroonilist haigust?

	Olen põdenud	**Põen käesoleval ajal**	**Ema või isa on põdenud**
Suhkrutõbi			
Maksahaigused			
Seedehäired/soolehaigused			
Kasvajad			
Südame haigused			
Liigeshaiguseid			
Närvisüsteemi haigused			
Neeruhaigused			
Verehaigused			
Kilpnäärme haigus			
Raske infektsioonhaigus			
Seljavalu (üle kuu)			
Midagi, mis nimetamata			

4. Kas olete pidanud lamama voodis rohkem kui kaks kuud järjest?

EI	**JAH**

Kui **JAH**, siis miks? _____

5. Kas Te olete viimase 2 nädala jooksul pidanud lamama voodis rohkem kui 2 päeva järjest?

EI	**JAH**

Kui **JAH**, siis miks? _____

15

6. Kas Te olete kasutanud või kasutate juba pikemat aega (üle 2 kuu) mõnda ravimit?

EI	JAH

Kasv, kaal ja toitumine

7. Kui pikk olite 25 aastaselt? _____

8. Kui palju Te kaalusite 25 aastaselt?

9. Mitu klaasi rõõska piima tarbite päevas keskmiselt? _____

10. Lapsena jõite rõõska piima

Palju	Keskmiselt	Mõnikord	Ei joonud

11. Kas rõõsk piim põhjustab Teile mingeid kõhuvaevusi?

EI	JAH

Kui **JAH** siis

Vaevus	Sagedus		
	Alati	Mõnikord	Harva
	Alati	Mõnikord	Harva
	Alati	Mõnikord	Harva

12. Kui tihti tarvitate teisi piimaprodukte?

Iga päev	Paaril korral nädalas	Ei tarvita üldse

Küsimused naistele (mehed jätkavad küsimusega nr. 18)

13. Kui vanalt algasid Teil menstruatsioonid? _____

14. Kas Te kasutate või olete kasutanud rasestumisvastaseid pille või muid hormonaalseid rasestumisvastaseid vahendeid.

EI	JAH

Kui JAH, siis kui pikka aega olete neid kasutanud? _____

13. Mitu last Teil on? _____

14. Mitu rasedust on Teil olnud? _____

15. Mitut last olete imetanud kauem kui 3 kuud? _____

16. Millal oli Teie viimane menstruatsioon? _____

17. Kas menstruatsioonid on/olid regulaarsed?

EI	JAH

Kohv ja alkohol

18. Kui sageli Te tarvitate alkoholi?

Ei tarvita	Kord kuus või harvem	2 kuni 4 korda kuus	2-3 korda nädalas	4 või enam korda nädalas

19. Mitu **drinki** Te tavaliselt korraga joote?

1 drink on:

- 1 pudel õlut või siidrit
- 1 klaas veini (12cl)
- 1 pits kanget alkoholi (4cl)

1-2 drinki	3-4 drinki	5-6 drinki	7-9 drinki	Üle 10 dringi

20. Kui sageli Te joote kohvi?

Ei joo üldse	Iga päev ei joo	1-2 tassi päevas	3-4 tassi päevas	Üle 4 tassi päevas

21. Kui vanalt Te alustasite kohvi joomist? _____

22. Kohvi eelistate

mustalt	koorega	piimaga

Suitsetamine

23. Kas Te suitsetate?

EI	**JAH**

24. Olete varem suitsetanud?

EI	**JAH**

25. Kui vanalt Te alustasite suitsetamist? _____

26. Kui pikk on/oli Teie suitsetaja staaz? _____

27. Mitu pakki Te keskmiselt päevas suitsetate/suitsetasite? _____

Eluviis

28. Suvel päikese käes tavaliselt

päevitate kogu keha	päevitate vaid käsivarsi, kaela ja sääri	väldite päevitamist teadlikult	kasutate alati päikesekaitset

Kui väldite päevitamist, siis miks? _____

29. Võrreldes teistega hakkab päike teile peale

hästi	sama moodi	hakkab halvasti peale

30. Mitmel päeval nädalas tegelete **raske** füüsilise koormusega nt. raskuste tõstmine, kaevamine, treening (jooksmine, kiire jalgrattaga sõit jne)?

ei ole rasket füüsilist koormust	_____ päeval nädalas	→ tundi päevas_____

31. Mitmel päeval nädalas tegelete **mõõduka** füüsilise koormusega? Mõõdukas füüsiline koormus paneb kiiremini hingama kui normaalne (näiteks kergete raskuste tõstmine, jalgrattaga sõit jne.).

ei ole mõõdukat füüsilist koormust	_____ päeval nädalas	→ tundi päevas_____

32. Mitmel päeval nädalas **jalutate** vähemalt 10 minutit järjest (arvestades jalutamist tööl, kodus, ühest kohast teise minnes, sportlikel eesmärkidel või vabal ajal)?

ei jaluta üle 10 minuti korraga	_____ päeval nädalas	→ tundi päevas_____

33. Mitu tundi päevas Te istute (s.h töö juures, kodus, õppetööd tehes, vabal ajal, külas olles, lugedes või televiisorit vaadates)? _____

Luustikust

34. Kas Teil on olnud luumurde?

EI	**JAH**

Kui **JAH**,

millises piirkonnas ?	**kui vanalt?**

35. Kas Teie vanematel või õdedel-vendadel on olnud enne 45. eluaastat luumurde?

Ei ole	**Ei tea/ei mäleta**	**JAH**

Kui **JAH**, siis

Kellel?	Kui vanalt?

Seljavalu

36. Kas Teil on olnud seljavalu, mis on kestnud üle kuu?

EI	**JAH**

37. Kui **JAH**, siis kui sageli oli seljavalu möödunud nädalal?

Ei ole olnud valu	Mõnel päeval nädalas	Iga päev

Kui Teil on olnud seljavalu, siis palun märkiga valu tugevus järgmisel skaalal

X_____X

Ei ole valu — väljakannatamatu valu

Kõhuvaevused

38. Kui sageli esineb Teil järgmisi vaevusi?

	Iga päev	**Vähemalt 1 kord nädalas**	**Vähemalt 1 kord kuus**	**Harvem kui 1 kord kuus**	**Ei esine**
kõhuvaevusi					
kõrvetisi					
röhatisi					
kõhuvalu					
kõhupuhitusi					
kõhukorisemist					
iiveldust					
oksendamist					
varajast küllastustunnet					
kõhulahtisust					
kõhukinnisust					
roojapidamatust					

39. Millised järgmistest vaevustest on Teil esinenud viimase kuu aja jooksul ja kui tugevalt?

	Viimase kuu jooksul	**Vaevuse tugevus** 1 - vähene (vaevust võib ignoreerida) 2 - keskmine (vaevust ei saa ignoreerida, kuid ta ei sega igapäevaseid tegevusi) 3 - äge, oluline (häirib igapäevast elu)	**Kui kaua kestis**
kõhuvaevusi			
kõrvetisi			
röhatisi			
kõhuvalu			
kõhupuhitusi			
kõhukorisemist			
iiveldust			
oksendamist			
varajast küllastustunnet			
kõhulahtisust			
kõhukinnisust			
roojapidamatust			

40. Kui sageli käib Teil kõht läbi? _____päevas/nädalas

Kasutatavad ravimid

Ravim	**Olen kasutanud**	**Kasutan**	**Märkused**
Diureetikumid			
Antatsiidid			
Meessuguhormoonid			
Kaltsitoniin			
Ca2+/vit.D (Calcigran)			
Östrogeenid			
Kortikosteroidid			
Antikoagulandid			
Bisfosfonaadid			
Parkinsoni ravimid			
Kasvaja ravimid			
Tsütostaatikumid			
Isoniasiid			
Liitium			
Kasvuhormoon			
Muud			

Biomeetria

Dünamomeetria	1. katse	2.katse	3.katse
Vasak käsi			
Parem käsi			
Pikkus			
Kaal			

PUBLICATIONS

CURRICULUM VITAE

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2006– …	Residency training in rheumatology, University of Tartu, Department of Internal Medicine

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AMGEN Bone Academy
American Society of Bone and Mineral Research (ASBMR)

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Main areas of research include the epidemiologic aspects of osteoporosis in Estonia, the role of vitamin D and other factors (incl. adult type hypolactasia) to bone mineral metabolism. Ten publications have been published (six in international peer-reviewed journals and 4 in Eesti Arst). Several theses have been published in the leading scientific meetings of the field.

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2006– …	Arst-resident reumatoloogia erialal, SA TÜK Sisekliinik

Ühiskondlik tegevus

Eesti Reumatoloogia Seltsi liige
AMGEN Bone Academy liige
American Society of Bone and Mineral Research liige

Teaduslik tegevus

Peamisteks uurimissuundadeks on osteoporoosi epidemioloogilised aspektid eestis, D-vitamiini mõju organismile ja luudele. Samuti teiste tegurite (s.h pärilik täiskasvanute hüpolaktaasia) mõju luude ainevahetusele. Ühtekokku on ilmunud 10 teaduspublikatsiooni, milledest 6 rahvusvahelistes eelretsenseeritavates teadusajakirjades ja 4 ajakirjas Eesti Arst. Poster- ja suuliste ettekannetega on esinetud mitmetel eriala juhtivatel teaduskonverentsidel.

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