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**Associations of lactase and apolipoprotein E gene polymorphisms and
physical activity with peripheral bone traits**

ACADEMIC DISSERTATION

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ABSTRACT

Previous findings suggest that bone mineral density and bone loss are highly genetically determined but the further details of bone genetics remain partly unknown. In thesis, it was studied for the first time whether the single nucleotide polymorphisms of lactase and apolipoprotein E (APOE) genes are associated with the peripheral quantitative computed tomography (pQCT) bone traits of radius and tibia. In addition to the gene polymorphisms, the associations of physical activity in childhood and adulthood with various bone traits as outcomes were examined. This is also a current topic since despite the fact that bone-loading physical activities are essential in building healthy bones; it seems that modern communities do not encourage most of us to move and exercise enough.

Study subjects of the present thesis are part of the Cardiovascular Risk in Young Finns Study which was initiated in 1980 with 3596 persons aged 3, 6, 9, 12, 15 and 18. The findings presented are additionally based on the follow-ups conducted in 1986, 2001 and 2007. The pQCT and quantitative ultrasound bone measurements and fracture data were collected in 2008 when the participants were 31-46 years old. Lactase C/T-13910 genotyping (*rs4988235*) was performed using a 5' nuclease assay and the APOE genotypes (*rs429358* and *rs7412*) and the APOE promoter polymorphisms -219 and +113 (*rs405509* and *rs440446*) using TaqMan SNP genotyping assays. Dietary intakes were collected through 48-hour dietary recall interviews and later in 2007 with a 131-item food frequency questionnaire. Physical activity in childhood was assessed using a questionnaire including items on leisure-time physical activity, organised exercise, participation in competitions, and intensity level. In adulthood, pedometer-determined steps were used to quantify the amount of physical activity undertaken in 2007.

In Study I, small differences in trabecular densities at the distal sites of radius and tibia were found in men between the lactase genotypes. Men with the T/T genotype had ~3% higher values than the carriers of T/C and C/C genotypes. They also had higher calcium intake than men carrying the C allele. In Study II, the carriers of the APOE ϵ 4 allele had higher total-cholesterol and LDL-cholesterol serum concentrations than the non-carriers. In addition, the ϵ 4 allele was associated with some lower cortical density but greater bone mineral content (BMC) at the tibial diaphysis. Women with the APOE promoter -219T/T allele had on average 7-8% lower cortical and compressive strengths at the distal sites of radius and tibia than the female G/G carriers. At the tibial shaft, the mean values of cortical strength were 2-2.5% lower in subjects with the -219T/T allele compared to the G/G carriers. Men with the -219T/T allele also had 1-2% lower cortical density and cortical strength at the radial shaft but 4-5% greater total areas at the radius than men with the G/G allele. In addition, women with the -219T/T allele whose longitudinal saturated fat intake was the highest (≥ 35.5 g/day) had the lowest total area and torsional strength at the radial shaft. Almost identical results were found in women and men with the +113G/C genotypes (G/G, G/C and C/C) as reported for the -219G/T genotypes (G/G, G/T and T/T). In Study III, there were no statistically significant differences in the risk of low-energy fractures between the different physical activity groups of 3-18-year-old children and adolescents. In addition, no differences were found in adult tibial traits between the physical activity groups of 3-6-year-olds. In females, the

highest level of physical activity at the age of 9-18 years was significantly associated with a lower likelihood of below median values of compressive strength at the distal tibia, and total and cortical areas, and BMC and strength indices at the tibial shaft compared to the lowest activity level in adolescence (ORs 0.33-0.53). Similarly in males, total area at the distal site and cortical area and strength at the tibial shaft were less likely to be below the median values in the highest physical activity group compared to the lowest activity group of adolescents (ORs 0.48-0.53). In Study IV, women within the highest tertile of daily steps had 3.8% and 0.5% greater broadband ultrasound attenuation and speed of sound at the calcaneus compared to women in the lowest tertile. In tibia, women in the highest tertile (> 8765 daily steps) had on average 1-5.4% greater bone cross-sectional area, BMC, trabecular density and compressive strength at the distal site and 1.6-2.7% greater total and cortical bone areas, BMC and torsional strength at the shaft compared to their study peers. Similarly, BMC and BSI at the distal radius and bone areas, BMC and torsional strength at radial shaft were 1.7-3.4% greater in women within the highest tertile of daily steps compared to the women with a lower number of daily steps. In men, the differences in studied bone traits were mainly non-significant across the tertiles of daily steps. Statistically significant results presented have p-values of ≤ 0.05 .

In conclusion, the genetic heterogeneity in lactase gene or the APOE $\epsilon 2/\epsilon 3/\epsilon 4$ genetic variation seemed to have little effect on the studied bone traits at the radius and tibia. Instead, the APOE promoter -219T/T and +113C/C alleles were associated with lower cortical bone phenotypes in both genders compared to the G/G carriers. In women, these supposed risk alleles of APOE promoter polymorphisms were also associated with lower total area and torsional strength at the radial shaft when the intake of saturated fat was high. In addition, a high level of physical activity at the age of 9-18 years, but not in younger children, was associated with wider and stronger weight-bearing tibia in both females and males. In adulthood, a higher amount of physical activity measured as daily steps was associated with greater bone cross-sectional area, mineral mass and strength at the calcaneus, tibia and radius in women.

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LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original articles referred to in the text by their Roman numerals:

- I Tolonen S, Laaksonen M, Mikkilä V, Sievänen H, Mononen N, Räsänen L, Viikari J, Raitakari OT, Kähönen M, Lehtimäki TJ. Lactase gene C/T⁻¹³⁹¹⁰ polymorphism, calcium intake, and pQCT bone traits in Finnish adults. *Calcif Tissue Int* 2011; 88: 153-161.

- II Tolonen S, Mikkilä V, Laaksonen M, Sievänen H, Mononen N, Hernesniemi J, Vehkalahti K, Viikari J, Raitakari O, Kähönen M, Lehtimäki T. Association of apolipoprotein E promoter polymorphisms with bone structural traits is modified by dietary saturated fat intake – The Cardiovascular Risk in Young Finns Study. *Bone* 2011; 48: 1058-1065.

- III Tolonen S, Sievänen H, Mikkilä V, Telama R, Oikonen M, Laaksonen M, Viikari J, Kähönen M, Raitakari OT. Adolescence physical activity is associated with higher tibial pQCT bone values in adulthood after 28-years of follow-up – The Cardiovascular Risk in Young Finns Study. *Bone* 2015; 75: 77-83.

- IV Tolonen S, Sievänen H, Hirvensalo M, Laaksonen M, Mikkilä V, Pälve K, Lehtimäki T, Raitakari O, Kähönen M. Higher step count is associated with greater bone mass and strength in women but not in men. *Arch Osteoporos* 2018; 13:20.

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1. INTRODUCTION

Why should we study bones? My answer is that we should do so because bones themselves and their adaptation to the environment are interesting. Bones grow throughout the ageing process and are estimated to achieve the highest bone mineral mass during the individual's early 20s. Bones are constantly deposited and remodelled, and in some phases of life they reach the point where more bone is lost than is made. Porous, osteoporotic bone is unfortunately a common health condition in women and men over 50 years of age. The first sign of this 'silent disease' is often a bone fracture which can then cause disabling effects for their carriers, such as intense pain or loss of independence. In addition to individual burden, osteoporosis and fractures also add to the financial costs borne by society as a whole. In the European Union, the costs of osteoporosis were estimated to be as high as 37 billion euros in 2010 and most of the costs came from treating fractures (Hernlund et al. 2013). The total costs of osteoporosis were, however, evaluated to be much higher and are expected to increase in the future.

The population studied is based on a multi-centre Finnish population that has been followed since 1980, when the study participants were 3, 6, 9, 12, 15 and 18 years old. In the latest study, taking place approximately 30 years later, these children had reached the age of 33-50 years. In the bone field, this is a unique population including both women and men and comprehensive information about their health, lifestyle, heredity, fractures and peripheral bones which was particularly of interest in the present thesis.

A candidate gene approach was used to study the associations of lactase and apolipoprotein E (APOE) gene polymorphisms with the pQCT-measured bone phenotypes of radius and tibia. The lactase gene C/T-13910 polymorphism is known to affect lactase-phlorizin hydrolase activity in the small intestine and thereby the digestion of lactose-containing foods and beverages. Subjects with the C/C-13910 variant have a phenotype of adult lactase non-persistence and according to previous evidence they use less milk and get less calcium from their diets than subjects with the C/T and T/T genotypes. This may predispose them to lower bone mass and greater incidence of fractures.

In addition, the APOE genetic variants $\epsilon 2$, $\epsilon 3$ and $\epsilon 4$ have been associated with bone health. APOE protein participates in the removal of lipoproteins and chylomicron remnants from plasma to several tissues serving as a ligand for the receptors. This influences plasma lipid levels so that the carriers of $\epsilon 2$ allele typically have the lowest and the carriers of $\epsilon 4$ allele the highest cholesterol blood concentrations. The lipid levels of subjects with the $\epsilon 4$ allele have been shown to be modulated by dietary fat and cholesterol interventions. It is suggested that unfavourable lipid profile in the carriers of $\epsilon 4$ allele may predispose them to lower bone mineral mass. In addition to the common APOE genotypes, the single nucleotide polymorphisms -219G/T and +113G/C in the promoter area of APOE gene were studied along with the pQCT bone traits. Previous studies have linked these two promoter polymorphisms to cardiovascular diseases; however, bone studies are lacking.

In addition to genetics, different lifestyle factors influence our skeletal traits and structure. In this thesis, we studied whether the skeletal benefits of physical activity during childhood and adolescence could be seen in adult tibial bone traits many years later. According to previous evidence, it seems that the early years of life are crucial to exercise-induced bone accrual in loaded bones to such an extent that is no longer attained at a mature age. Both children and adults are recommended to move and exercise on a daily basis but on average only one third of children and one fifth of adults reach the current recommendations for physical activity in Finland. Recommended bone-enhancing activities include, for instance, sports or activities involving running or brisk walking, rapid turns and jumps. In addition to the effects of exercise on bones during childhood and adolescence, it was studied here whether physical activity quantified as daily steps is associated with different peripheral bone traits in adulthood. Pedometer-determined steps perhaps provided a more objective method for measuring physical activity than a questionnaire; though, as a scientific method it is not without limitations.

2. REVIEW OF THE LITERATURE

2.1 Structure and shape of long bones

Bone is principally made up of fibrous type I collagen and minerals (mainly calcium phosphate), water, and also includes living cells and blood vessels (Currey 2006). Many bones also contain hematopoietic marrow and a thin layer of cartilage often exists at the end of the bone. Proteins in the bone other than collagen are simply called noncollagenous proteins (NCPs), which account for approximately 10-15% of all proteins in bone.

Bone cells are called osteoblasts, osteocytes and osteoclasts. Osteoblasts derive from bone lining cells ('quiescent osteoblasts') and participate in bone formation. Osteocytes are bone cells in the bone tissue and derive from osteoblasts. They connect with other osteocytes and with bone lining cells via canaliculi and gap junctions. Meanwhile, osteoclasts are bone destroying cells and are made of precursor cells circulating in the blood.

Woven and lamellar bone tissues differ from each other in how fast they are made and how collagen fibrils and mineral crystals are oriented. Lamellar bone is more precisely arranged but less mineralized than woven bone. Lamellar bone is also laid down more slowly and is, for example, found in Haversian bone (secondary osteons) where osteoclasts form a cutting cone on the bone which then begins to be filled in again by osteoblasts. In addition to woven and lamellar bone, there is a parallel-fibred bone which has a structure that is intermediate between these two bone tissues. Woven bone is more common in fibrolamellar bone than lamellar bone and Haversian bone. Fibrolamellar bone exists in bones that are growing quickly and it contains more minerals than lamellar bone.

In addition to these previously mentioned bone tissues, there are two main bone structures called compact and cancellous bone (or cortical and trabecular bone). Cancellous bone has large spaces and often contains blood vessels. In adults, it is primary lamellar or Haversian bone but in growing bones cancellous bone can also be made of woven or parallel-fibred bone. Long bones, like the radius and tibia, include cancellous bone at their ends with a thin layer of compact bone on it. Compact bone has spaces only for osteocytes, canaliculi, blood vessels and erosion cavities.

Long bones grow in length at their epiphyseal plates which are placed at the ends of these bones. This phenomenon is called endochondral ossification where calcified cartilage is replaced by bone. The shape of radius and tibia is hollow and the section is often circular, especially at the shaft part. Long bones expand at their ends and are capped with synovial cartilage, which connects the bones and reduces pain and stress in the joints. Expanded ends of long bones are filled with cancellous bone which is covered by a thin sheet of compact bone. In addition to having a tubular shape, long bones have flanges and tubercles for the attachment of muscles and ligaments. Human long bones of forearm and lower leg are shown in Figures 1 and 2 below.

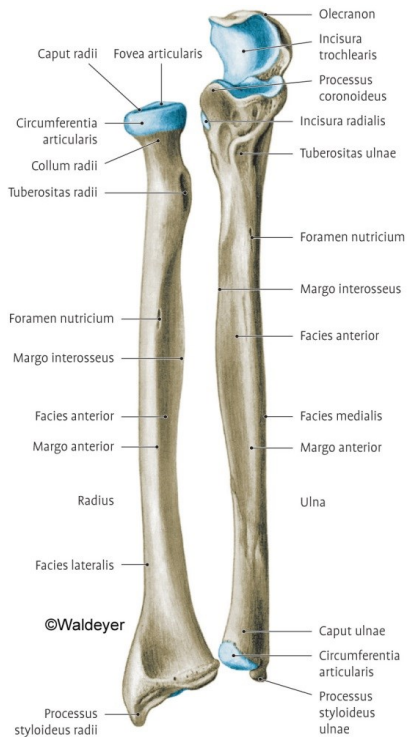


Figure 1. Ventral picture of radius and ulna from the right forearm (©Kustannus Oy Duodecim).

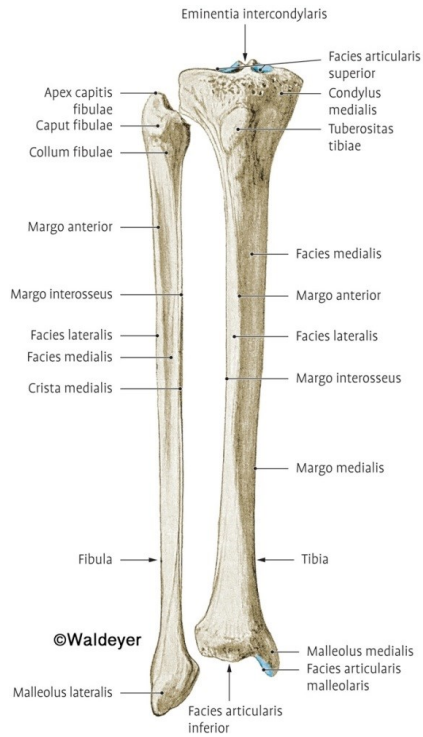


Figure 2. Ventral picture of fibula and tibia from the right lower leg (©Kustannus Oy Duodecim).

2.1.1 Osteoporosis and general risk factors of bone deformation

Studying peripheral long bones, such as the radius and tibia, is important because in real life these parts of the body are often subject to fractures. In fact, fractures at the lower part of the radius are the most common fracture in upper limbs (Fracture at the lower part of radius (wrist fracture): Current Care Guidelines Abstract 2016). It is estimated that 12 000 fractures at the lower part of the radius occur annually in Finland. The incidence of radial fractures is known to increase with ageing. Fractures in the lower leg are also quite common, especially in 10-19-year-old men and older women (Fractures in the tibia: Current Care Guidelines Abstract 2011).

In osteoporotic bone, the risk of fractures is increased and fractures can also be the first symptom of osteoporosis (International Osteoporosis Foundation, IOF, 2016). Living bone tissue goes through constant changes and it is estimated that the highest bone mass is reached during the early 20s. During the ageing process bone is dissolved and deposited, and when more bone is lost than made, bone becomes porous and brittle. Unfortunately, osteoporosis is a common bone disease. According to IOF, one in three women and one in five men over the age of fifty are at risk of an osteoporotic fracture worldwide. Osteoporotic fractures occurring at the hip, spine and wrist can have serious consequences such as intense back pain, loss of independence or even death. According to the World Health Organization, osteoporosis is defined as bone mineral density (BMD) equal to or more than 2.5 SD below the reference value of young healthy adults (same as T-

score of -2.5 or lower) (WHO 1994). Reference measurements of osteoporosis are based on dual-energy X-ray absorptiometry (DXA) scans. If BMD cannot be measured or one already knows her or his BMD at the femoral neck, Finnish Current Care Guidelines recommend using the Fracture Risk Assessment Tool (FRAX®) before starting a bone medicine program (Osteoporosis: Current Care Guidelines Abstract 2018). The FRAX® tool gives an estimate for the 10-year probability of a major osteoporotic fracture and is available on the internet:

<http://www.shef.ac.uk/FRAX/index.aspx>.

Many factors can reduce bone mineral mass and cause primary or secondary osteoporosis (IOF 2016). Some of these factors cannot be changed, such as age or parental history of fracture. Secondary osteoporosis is caused by different medical disorders and treatments such as kidney failure, anorexia nervosa, rheumatoid arthritis, gastrointestinal diseases or different hormonal imbalances, all of which can cause increased bone loss. Additionally, some medications, such as long term glucocorticoid therapy, increase the risk of secondary osteoporosis. Modifiable risk factors are different lifestyle choices and conditions which are particularly useful in the prevention of fractures and osteoporosis. For instance, regular exercise, such as balance and strength training, can prevent fall-related fractures in old age as it can develop, maintain and restore physical functioning of individuals (Karinkanta et al. 2010).

In the Cardiovascular Risk in Young Finns Study population, Crohn's disease or ulcerative colitis, corticosteroid treatment and physical inactivity increased the risk of low trabecular bone mass density (BMD) at the distal radius (relative risks, RRs 1.34-2.43, p-values <0.05) (Laaksonen et al. 2010). Risk factors for low trabecular BMD at the distal tibia were underweight (body mass index, BMI < 19 kg/m²), epilepsy, excess alcohol intake (≥ 3 drinks/day) and history of smoking (RRs 1.29-2.95). Obesity (BMI > 30 kg/m²) seemed to decrease the risk of having a low BMD at the distal sites of both studied bones (RRs 0.30-0.45). The risk of low-energy fractures (at the age of ≥ 20 years) was associated with anorexia nervosa, excess alcohol intake and hypogonadism (ovarial or testicular insufficiency) (RRs 2.08-3.74).

2.1.2 Calcium and healthy bones

In the form of hydroxyapatite, calcium is one of the main minerals in bone and therefore its adequate intake from diet and in some cases from supplements should be ensured (Bonjour et al. 2009). Recommended intake of calcium that covers the requirements of most individuals varies from 540 mg to 900 mg per day (Nordic Nutrition Recommendations 2012). Females and males aged 10 years or older are recommended to consume 800-900 mg calcium/day. Almost all calcium in the adult body (~1200-1400 g) is found in the skeleton and teeth. The highest calcium accretion happens during pubertal growth and so-called peak bone mass, the highest bone mineral mass, is attained during late adolescence (Magarey et al. 1999, Weaver et al. 2016). Mean daily intakes of calcium exceed the recommended levels in Finnish adults mostly coming from milk products (Helldán et al. 2012). However, in adults aged 65-74 years who did not drink milk, the average intake of calcium was below the recommended intake (~698 mg/day in women and ~725 mg/day in men). In Finnish secondary school pupils who had an average age of 14 years, dietary calcium

intake per day was 1032 mg in girls and 1273 mg in boys (Hoppu et al. 2008). 97-99% of adolescents reported using milk products, which are the main source of calcium in Finnish diets.

Calcium (and phosphorus) homeostasis is mainly regulated in the intestine, bones and kidneys by parathyroid hormone (PTH) and calcitriol (1,25-(OH)₂D). If calcium concentration in blood decreases, PTH is secreted from parathyroid gland (Schmitt et al. 1996) and the active form of vitamin D (calcitriol) is mainly produced in the kidneys, which both stimulates calcium mobilization from bone and decreases renal calcium excretion to boost calcium levels back into the normal range (Wacker & Holick 2013). In addition to PTH, the renal synthesis of calcitriol is regulated by several factors, such as serum calcium and itself. Calcitriol also increases intestinal calcium absorption in the small intestine by promoting the expression of an epithelial calcium channel and a calcium binding protein.

2.2 Bone genetics

2.2.1 Genome wide association studies of bone mineral density

Table 1. Genome wide association studies of bone mineral density

Study and population origin	Initial sample	Replication sample	New SNP-BMD associations ¹
Richards et al. 2008, UK	2094 females	6463 individuals	2
Styrkarsdottir et al. 2008, Iceland, Denmark, Australia	5861 individuals	7925 individuals	14
Styrkarsdottir et al. 2009, Iceland, Denmark, Australia	6865 individuals	8510 individuals	15
Rivadeneira et al. 2009, Netherlands, UK	19195 individuals		29
Kung et al. 2010, China, UK	785 females	18098 individuals	1
Duncan et al. 2011, Australia, UK, New Zealand	1955 females	20898 females	9
Estrada et al. 2012, Finland, US, Iceland, Netherlands, UK, Ireland, East Asia	32961 individuals	50933 individuals	69
Medina-Gomez et al. 2012, Netherlands, Sweden, UK	1834 individuals	11052 individuals	2
Styrkarsdottir et al. 2013, Iceland	73965 individuals		2
Zhang et al. 2014, Europe, East Asia, US, Latin America	11140 individuals	15921 individuals	24
Styrkarsdottir et al. 2016, Iceland, East Asia	20162 individuals	10092 individuals	14

¹Detailed information on single nucleotide polymorphisms (SNPs) associated with bone mineral density (BMD) is found in the original articles.

Genome wide association studies (GWAS) involving hundreds of thousands of single nucleotide polymorphisms (SNPs) aim to identify associations with complex clinical conditions and phenotypic traits, hoping to thereby reveal something about the etiology of studied conditions and traits (Welter et al. 2014). The largest GWAS related to BMD are listed in Table 1 (Burdett et al. 2017). To date, researchers have identified hundreds of different SNPs as being associated with BMD. In the present thesis, the lactase C/T-13910 polymorphism (SNP *rs4988235*), the APOE (*rs429358* and *rs7412*) and the APOE promoter polymorphisms -219 and +113 (*rs405509* and *rs440446*) were selected because of their possible interactions with both dietary factors and bone traits. These specific SNPs are described in more detail in the next chapters 2.2.2 and 2.2.3.

2.2.2 Lactase gene C/T-13910 polymorphism, calcium and bone

The lactase gene C/T-13910 polymorphism, located 13910 base pairs from the 5' end of the lactase gene in chromosome 2, was found to be associated with lactase-phlorizin hydrolase (LPH) activity in the small intestine which hydrolyses lactose into glucose and galactose (Enattah et al. 2002). The homozygous variant C/C of the C/T-13910 polymorphism is characterised by a decline of intestinal lactase enzyme and a phenotype of lactase nonpersistence (lactose intolerance). Individuals with the T (-13910) allele have a higher expression of LPH mRNA than individuals with the C (-13910) allele, a phenomenon which is related to the transcriptional regulation of the LPH gene and elevation of lactase activity (Kuokkanen et al. 2003). This finding was supported by a functional cell line study where the T allele enhanced lactase-promoter activity more than the C variant (Troelsen et al. 2003). The variants of LCT polymorphism are also in clinical use as a screening test for lactose intolerance (Rasinperä et al. 2004).

Previous evidence links the C/T-13910 polymorphism to milk consumption, lower intake of calcium, bone mineral density and fractures. In a study of postmenopausal women, the carriers of C/C and T/C genotypes had 7-11% lower BMD at the hip and/or spine (Obermayer-Pietsch et al. 2004). The C/C (-13910) genotype was also associated with higher bone fracture incidence, lower calcium intake from milk and aversion to milk consumption, although, no differences were found in total calcium intake or in intestinal calcium absorption. In a study including elderly people, the C/C genotype was associated with greater risk of hip and wrist fractures (Enattah et al. 2005). In the previous study from the Young Finns cohort, females with the C/C-13910 genotype had lower milk product consumption and intakes of protein and calcium over the study years 1980-2001 compared to females with the other lactase genotypes (Lehtimäki et al. 2006). The consumption of milk products was also lowest among males with the C/C genotype over the study years, especially in those over 10 years of age. In the same cohort, it was later revealed that subjects with the C/C genotype used especially little milk but no differences were found in the use of other milk products (Laaksonen et al. 2009). In addition, inadequate calcium intake was more common in subjects with the C/C genotype. Similar results have also been found in a study including Swedish children and adolescents belonging to the European Youth Heart Study (Almon et al. 2013). In a study of postmenopausal women, the C/C genotype was again associated with aversion to milk consumption, and also with decreased serum calcium level and lower BMD (Z-score) at the radius, total hip and Ward's triangle when compared to the T/T and T/C genotypes (Bàcsi et al. 2009).

However, not all studies have confirmed the C/C-13910 variant as a potential risk factor for osteoporosis. In a study including young men, the LCT polymorphism was not related to calcium intake or BMCs, scan areas or BMDs at the different measurement sites of lumbar spine and upper femur (Enattah et al. 2004). In addition, the levels of serum 25-OH vitamin D, parathyroid hormone (iPTH) and the markers of bone turnover rate were similar across the lactase genotypes in the previous study. In another study setting, Enattah et al. (2005) reported that calcium intake from dairy products, BMD of the heel or the prevalence of fractures and osteoporosis did not differ between postmenopausal women genotyped for the LCT polymorphism. Furthermore, differences between the lactase genotypes in BMC and BMD at the lumbar spine and femoral neck were not found in the sub-cohort of the Young Finns (Laaksonen et al. 2009).

2.2.3 Apolipoprotein E gene polymorphisms, saturated fat and bone

In humans, the genetic polymorphism of apolipoprotein E (APOE) located in chromosome 19 is present in three common alleles ($\epsilon 2$, $\epsilon 3$, $\epsilon 4$) which code for three isoforms epsilon(E)2, E3 and E4 (Siest et al. 1995). The isoforms differ by cysteine-arginine interchanges at amino acid positions 112 and 158. At the genetic level, both codons include T to C nucleotide changes, contributing to the production of six genotypes E2/2, E2/3, E2/4, E3/3, E3/4 and E4/4. In lipid metabolism, APOE has a role in the transport of cholesterol and triglycerides, the metabolism of lipoprotein particles and the activation of lipolytic enzymes. APOE protein serves as a ligand for the low-density lipoprotein (LDL)-receptor and LDL-related receptor protein (LRP), controlling the removal of lipoproteins and chylomicron remnants from plasma to several tissues which then influence plasma cholesterol and triglyceride concentrations. The carriers of APOE $\epsilon 2$ allele have a defective affinity for the LDL-receptor which induces an upregulation of the liver LDL-receptor, leading to low plasma cholesterol concentrations. The situation in the carriers of APOE $\epsilon 4$ allele is the opposite; hence they have higher plasma cholesterol than carriers of $\epsilon 2$ allele do. Those with the $\epsilon 3$ allele usually have intermediate cholesterol levels compared to the carriers of the $\epsilon 2$ and $\epsilon 4$ alleles. Differences in LDL-cholesterol levels and coronary risk were also reported in a large meta-analysis where individuals with the $\epsilon 2$ allele had lower lipid levels and coronary disease risk compared to the two other groups (Bennet et al. 2007). In addition, it was found in the Young Finns Study that the polymorphism of APOE first begins to influence the lipid profile during childhood (Lehtimäki et al. 1990, Grönroos et al. 2007). Lehtimäki and others also suggested that the APOE variants may influence total serum and LDL cholesterol levels in new-borns and that after birth, levels are additionally influenced by environmental factors (Lehtimäki et al. 1994).

Since the genetic variation of APOE gene is well represented in the lipid metabolism, it is an ideal candidate for gene-environment-interaction studies. In earlier studies, subjects with the $\epsilon 4$ allele have shown the greatest response in LDL-cholesterol to dietary changes in which the amounts of saturated fat or cholesterol or both have been modified (Tikkanen et al. 1990, Lehtimäki et al. 1992, Lopez-Miranda et al. 1994, Dreon et al. 1995, Schaefer et al. 1997, Sarkkinen et al. 1998). However, Dreon et al. (1995) reported that the reduction in LDL-cholesterol during a low-fat diet was caused by a shift from large cholesterol-rich LDL particles to smaller, denser LDL particles rather than a reduction in LDL particle number.

Moreover, the interaction of APOE polymorphism and bone health may be a consequence of oxidised LDL and other lipid oxidation products which have been shown to inhibit the differentiation of preosteoblasts in a mouse cell line (Parhami et al. 1997) and to increase osteoclastogenesis in hyperlipidemic mice with a high-fat diet (Tintut et al. 2004). Besides oxidised lipids, T-lymphocytes, isolated from the bone marrow cells of mice on a high-fat diet, were shown to support osteoclastic differentiation of cells and induce upregulation of osteoclastogenic cytokine, receptor activator of NF- κ B ligand (RANKL), in T-lymphocytes (Graham et al. 2010). In previous studies which chiefly included postmenopausal women, carriers of the APOE ϵ 4 allele had a lower BMD at the lumbar spine, femoral neck or trochanter, lower (total body) BMC and greater risk of fractures and more severe vertebral deformities than the noncarriers (Shiraki et al. 1997, Kohlmeier et al. 1998, Cayley et al. 1999, Pluijm et al. 2002). However, in a meta-analysis mostly including peri- and postmenopausal women, a modest association between the APOE ϵ 4 allele and BMD was found at the trochanter and lumbar spine but not at the other sites of the hip or with fracture risk (Peter et al. 2011). Additionally, no association was found between the APOE genotypes and dual-energy X-ray absorptiometry (DXA)-measured BMD at the lumbar spine and femoral neck in the two large South Korean studies conducted on humans (Kim et al. 2016).

The genetic variation of the APOE gene includes also the APOE -219G/T and +113G/C promoter polymorphisms which have been shown to modulate the transcription of the APOE gene in hepatoma cells (Artiga et al. 1998, Viiri et al. 2008). Substitution of the -219T allele for the -219G allele and the +113G allele to the +113C allele increased transcriptional activity by lowering the affinity of the bound nuclear proteins within the promoter area. It is suggested that carriers of the -219T allele may be at increased disease risk since the T allele and its haplotypes have been associated with increased risk of myocardial infarction in a large case-control study including males (Lambert et al. 2000), and coronary atherosclerosis and insulin resistance in Finnish families with a family history of premature coronary heart disease (Viitanen et al. 2001) and higher longitudinal concentration of LDL-cholesterol in males during the 21 year follow-up (Viiri et al. 2006). However, in a study of middle-aged Finnish men, the homozygous G/G genotypes of -219G/T and +113G/C promoter polymorphisms and -219G/+113G/epsilon3 haplotype were associated with unfavourable lipid and lipoprotein profiles (Viiri et al. 2005). Furthermore, in another study, -219G and +113G alleles were identified as genetic risk factors for ischemic strokes (Abboud et al. 2008).

2.3 Physical activity and bones

2.3.1 Children and adolescents

Previous evidence emphasises that muscular contractions during weight-bearing activities can site-specifically increase bone mass and size, especially in children who are either pre-pubertal or in the early stages of puberty (Strong et al. 2005). Dynamic mechanical forces exerted on load-bearing bones during typical physical activities influence bone's strength by either modelling or remodelling the bone tissue (Currey 2006, Frost 2003). In addition, microdamage in bone can to some extent increase bone strength if not accumulated. The strain-dependent threshold ranges of

bone determine which of the functions are predominant. It is not yet known which (bone) cells are responsible for these strain-dependent thresholds but they may be genetically determined. In addition to the mechanical feedback of bone, non-mechanical factors such as genes, hormones and calcium contribute to this system (Frost 2003). In young subjects, for instance, exercise-induced periosteal bone formation is suggested partly due to the enhancing effect of physical activity on growth hormone and insulin-like growth factor-I levels (Wang & Seeman 2008).

The amount of physical activity recommended for school-age children is 60 minutes or more of moderate-to-vigorous physical activity on a daily basis (Strong et al. 2005, Tammelin & Karvinen 2008). At the same time children and adolescents should be discouraged from sedentary activities such as excess television viewing or internet use. In a Finnish recommendation for bone-enhancing exercise, children and adolescents are recommended to participate in bone-loading activities and sports such as ball games, track and field sports, gymnastics and different children's games for approximately 60 minutes 3 times a week (Nikander et al. 2006).

In a large Finnish physical activity study, it was revealed that 31% of children and adolescents aged 9-15 engaged in moderate-to-vigorous physical activity for at least one hour a day (Kokko & Mehtälä 2016). The proportion of those who reached the physical activity recommendation was smaller among 13- and 15-year-olds (26% and 17% of children and adolescents, respectively) than among 9- and 11-year-olds (~40% in both). It was also found that only 5% of these children and adolescents spent less than 2 hours/day with a TV, computer, smartphone or other electronic devices, which is the current maximum recommended length of 'screen time'. In this previous study, in addition to using a questionnaire, physical activity was measured with an accelerometer. According to the accelerometer data, the amount of physical activity was greater among 9- and 11-year-olds than in older age groups, supporting the questionnaire results. Physical activity was mainly light in both genders and in all age groups but boys had more moderate-to-vigorous physical activity than girls. Children and adolescents took approximately 10 243 steps/day, which decreased in 13- and 15-year-olds. However, 47-60% of the waking time was measured as being spent in passive sitting or lying positions. According to the accelerometer data, the proportion of those who reached the recommendation of one hour of moderate-to-vigorous physical activity a day were 51% of 9-year-olds, 37% of 11-year-olds, 21% of 13-year-olds and 11% of 15-year-olds. Similar trends of decreasing physical activity in school-aged children by age have been noticed in different countries across Europe and North America (Currie et al. 2012). In addition, boys in general were more likely to meet the recommended level of daily physical activity than girls were, and that level of family affluence also modestly affected children's activity levels. In the School Health Promotion study of Finnish adolescents aged 14-20, the proportion of those in the 8th and 9th grades of comprehensive schools who exercised for a maximum of one hour per week during their leisure-time had decreased from 41% to 32% in the years 2000-2013 (Luopa et al. 2014). In first and second year students of upper secondary schools, the trend was similar to that in comprehensive schools. However, among the first and second year students of vocational schools, the proportion of those who exercised during their leisure-time for no more than one hour a week was higher than in upper secondary schools (47% of vocational students vs. 29% of secondary school students in 2013). In addition, 20-32% of the adolescents spent 4 hours or more on a daily basis with a TV, computer, mobile phone or other electronic device on weekdays in 2010-2013.

The benefits of physical activity on growing bones has previously been shown in the playing arms of tennis and squash players who started their playing before or at menarche compared to peers with initiation age after menarche (Kannus et al. 1995). In a similar study examining pQCT, the loading-induced differences in mass, size and torsional strength of humeral shaft, and bone mineral mass of distal radius were greatest in women who had started their playing before or at menarche (Kontulainen et al. 2002). In a review and meta-analysis of randomised controlled trials that studied the effect of long-term supervised exercise on whole bone strength in the lower-extremities, significant exercise effect was found only among pre-pubertal boys (Nikander et al. 2010). Bone strength was evaluated to increase 1-8% at the loaded skeletal sites when compared to habitually active or sedentary controls. In another review it was noted that weight-bearing activities enhance bone mineral accrual more in pre-pubertal children than during the pubertal stage (Hind & Burrows 2007).

In addition to the intervention studies, the skeletal benefits of childhood physical activity have been shown in prospective and retrospective studies with longer follow-ups (Lloyd et al. 2000, Baxter-Jones et al. 2008, Duckham et al. 2014, Nilsson et al. 2014). In females aged 12 years at the beginning of the study, the cumulative 6-year sports-exercise scores were positively associated with hip BMD at the age of 18 years (Lloyd et al. 2000). In a study of Baxter-Jones et al. (2008), adolescents with the highest physical activity scores during childhood and adolescence had 8-10% greater total hip and femoral neck BMC at the age of 23-30 years. These adolescents were additionally invited to pQCT measurements as young adults (Duckham et al. 2014). Physical activity in adolescence was positively associated with bone size at the tibial diaphysis in both females and males. Compared to their inactive peers, active adolescent males had 13% greater bone strength at the tibial shaft in young adulthood and active adolescent females had 12% and 3% more cortical and trabecular bone at the tibia, respectively. There is also some evidence that the skeletal benefits of physical activity at an early age may not disappear during later life as shown in a study where a competitive level of exercise at the age of 10-30 years was still associated with greater cortical bone size and estimated bone strength at the weight-bearing tibia in old age (Nilsson et al. 2014).

Several exercise intervention studies have used pQCT-derived bone traits as outcomes (Detter et al. 2014, Macdonald et al. 2007, Heinonen et al. 2000). In a 6-year school-based exercise intervention study, one of the four included elementary schools increased the amount of physical educational classes from the standard of 60 minutes/week to 40 minutes every school day (Detter et al. 2014). Classes included a variety of activities such as ball games, jumping and playing activities supervised by the usual teachers. In a subsample of these 6-9-year-old children, at the beginning, different bone traits were evaluated with the DXA, and then also with the pQCT at the follow-up. Girls in the intervention group gained more bone mass and greater bone size at the femoral neck and tibia than controls. And both girls and boys with additional physical education classes gained more bone mineral at the spine. This school-based exercise intervention program was later continued, and in the last intervention year, the annual fracture incidence was 52% lower in the intervention group compared with controls (Cöster et al. 2017). In another school-based intervention study with an equally long follow-up as the aforementioned study, a 7-month exercise intervention was reported to increase hip BMC in pre-pubertal children compared with

controls (Gunter et al. 2008). The exercise intervention included either a jumping (intervention group) or stretching (controls) program 3 days a week for 20 minutes per session. In a study of Macdonald et al. (2007), greater increase in bone strength at the distal tibia was found in pre-pubertal boys compared to controls after the median follow-up of 14 months. In addition to weekly physical education classes, boys and girls in the intervention groups took part in 15 minutes of extra physical activity every school day and a short activity of jumps three times a day on 4 days per week. In a Finnish 9-month high-impact exercise intervention study including two 50 minute length instructed workouts per week, premenarcheal girls gained more BMC at the lumbar spine and femoral neck than controls, whereas no significant differences were found in postmenarcheal girls or in cortical bone measured with the pQCT (Heinonen et al. 2000).

2.3.2 Adults

Maintaining bone mineral is one of the health goals of physical activity in adulthood, and likely results from attenuated bone resorption rather than a large increase in bone mass (Kohrt et al. 2004). It is suggested that bone mineral production starts to decrease after the age of 40, and particularly in women at the menopause, when the production of sex hormones decreases. In both genders, ageing causes bone loss mainly in the trabecular and endocortical bone of the endosteal surface (Frost 2003). In adults, the exercise prescription for bone health includes participating in weight-bearing endurance activities 3-5 times a week (e.g. jogging, walking, stair climbing) in addition to resistance training 2-3 times/week (weight lifting) and activities that involve jumps (e.g. ball games). Kohrt and others recommend that exercise programs for older people should additionally include activities that improve balance which may thereby prevent injurious falls and fractures. A Finnish recommendation for bone exercise of adults aged 18-50 is very similar to the recommendation of the American College of Sports Medicine presented above (Nikander et al. 2006). The recommended daily number of jumps is 50-100, which can be divided into several bouts. The current recommendation for health-enhancing physical activity for adults aged 18-64 is at least 2 hours and 30 minutes of moderate or 1 hour and 15 minutes of vigorous aerobic exercise per week such as walking, cycling, stair climbing, swimming and racket games (Terveysliikunnan Suositukset 2009). In addition, it is recommended that adults perform exercises that improve muscle strength and balance at least 2 times a week.

In a health survey of Finns aged 15-64, the proportion of those who exercised a minimum of 30 minutes at least four times a week was ~34% (Helldán & Helakorpi 2014). Their proportion has stayed rather stable after 1995 when the question was included. In addition, the proportion of those who either walked or cycled for at least 15 minutes a day to and from work was ~29%. In another Finnish health survey, it was revealed that ~21% of Finnish adults over 20 years of age did not exercise every week during their spare time (Murto et al. 2017). The proportion of passive individuals was greater among men than women (24.8% vs. 18%) and among older age groups and those with less education. These trends stayed very similar between the years 2013-2016. The sedentary behaviour and physical activity of 18-85-year-old Finnish adults in a sub-population of the Health 2011 Study were studied using an accelerometer (Husu et al. 2016). It was reported that 59% of waking time was passive, mainly involving sitting. Passive hours were high among all

participants, including those who reached the recommended level of health-enhancing physical activity and those who had a higher number of daily steps. The proportion of physical activity was less than 25% of the waking time, of which 15% was light activity. Husu and others concluded that from a health perspective we should find ways to decrease daily sedentary time and increase levels of physical activity in the general population.

Walking is one of the most common physical activities in humans and it has been previously suggested to increase bone mass and strength in older adults and children. In studies of postmenopausal Japanese women, relatively high counts of daily walking steps were positively associated with the ultrasound parameters of calcaneus and negatively associated with bone resorption (Kitagawa et al. 2003, Kitagawa & Nakahara 2008). In a population-based study of older women and men, those with more daily steps tended to have higher bone mineral density in the hips after the age of 65 years (Foley et al. 2010). In addition, in a randomised controlled trial of 52-53-year-old women, endurance training consisting mainly of brisk walking maintained more bone mineral at the femoral neck compared to the control group (Heinonen et al. 1998). Over a period of 18 months, endurance activities were performed approximately 3 times a week for 50 minutes per session, including a 10-minute warm-up, 30 minutes training and 10-minute cool-down. In a longitudinal study of Japanese elderly, daily steps were collected continuously for 5 years and subjects were classified according to the number of steps into four different groups each year (Shephard et al. 2017). The two highest groups of steps maintained their calcaneal bone stiffness during the follow-up whereas the calcaneal bone values of those in the two lowest quartiles decreased. Shephard et al. concluded that seniors taking at least 7000-8000 steps/day had optimal bone health. They also recommended physical activity at a moderate intensity level (> 3 METs) for 15-20 minutes/day. In addition to walking, other exercises involving ground reaction forces have had beneficial skeletal effects (Nikander et al. 2010). Female athletes participating in sports classified as high-impact, odd-impact or repetitive, low-impact exercise loadings (e.g. volleyball, triple jump, soccer, tennis and running) had on average 15-50% greater cortical bone area and bone strength at the tibia than the referents. However, different types of osteogenic stimulus may be needed in other bones such as the proximal femur (Nikander et al. 2009).

3. AIMS OF THE STUDY

Genetic and lifestyle factors are known to modulate skeletal traits such as bone mineral density and content but little is known about their associations with quantitative computed tomography (QCT) bone traits such as cortical bone and strength properties. In addition to the four gene polymorphisms of lactase and apolipoprotein E, which have not previously been studied in relation to the peripheral QCT bone traits of radius and tibia, the skeletal benefits of children's and adult's physical activity on adult tibial traits were examined.

The aims of this thesis were to investigate the following:

1. Whether single nucleotide polymorphism of lactase enzyme is associated with the pQCT bone traits of radius and tibia or the prevalence of low-energy fractures in this relatively healthy population of women and men aged 31-46 years (Study I). Additionally, the interactions of lactase genotypes and calcium intake in relation to the peripheral bone phenotypes were tested.
2. Whether radial and tibial bone traits are associated with the APOE ϵ 4 allele or with the APOE -219G/T and +113G/C promoter polymorphisms. Additionally, the interactions of these APOE gene polymorphisms and dietary longitudinal saturated fat intake with the pQCT bone traits were investigated (Study II).
3. Whether physical activity at the age of 3-18 years predicts the pQCT-measured bone phenotypes of weight-bearing tibia or the prevalence of low-energy fractures in adulthood (Study III).
4. Whether daily steps measured with a pedometer modify the quantitative ultrasound (QUS) bone traits of calcaneus and the pQCT bone traits of tibia and radius in the present population (Study IV).

4. POPULATION AND METHODS

4.1 Population

Participants in the present thesis were drawn from the Cardiovascular Risk in Young Finns Study (the Young Finns cohort) carried out by the universities and university hospitals of Turku, Helsinki, Tampere, Kuopio and Oulu (Raitakari et al. 2008). In the latest survey conducted in 2011-2012, participants from Jyväskylä were also included. The first baseline study was carried out in 1980, in which a total of 3596 persons aged 3, 6, 9, 12, 15 and 18 years participated (6 age cohorts). These subjects were randomly chosen from the national population register and the participation rate was 83.2% at the first survey. After the year 1980, seven larger follow-up studies were conducted and the same subjects were invited to the re-examinations in 1983, 1986, 1989, 2001, 2007 and 2012. The participation rates in the follow-up studies have varied from around 60 to 80%. The information used in this thesis was gathered in 1980, 1986, 2001 and 2007 (Studies I-IV).

Some background characteristics of the study subjects are shown in Table 2. In 2007, the average age was 37.7 years. Study subjects were slightly over the normal body mass index as women had the average BMI 25.4 and men 26.8. There were three times more underweight women than men (2.9% vs. 1%, p-value 0.002) but within the group of overweight individuals there was no significant difference between women and men (16.3% vs. 18.4%, p-value 0.19). The intake of energy, protein, dietary calcium and vitamin D were higher among men but the average proportion of protein in comparison with total energy intake tended to be the same in both groups. 25-hydroxycholecalciferol concentrations were, instead, higher among women (60.9 vs. 56.7 nmol/l, p-value <0.001). Alcohol consumption was higher among men and there were considerably more men who drank more than 3 drinks per day (1.6% of women vs. 12.1% of men, p-value <0.001). Smoking was rather common in the present population since 37.3% of women and 50.5% of men had smoked for at least one year during their lifetime. 14.9% of women and 22.8% of men were also current smokers in 2007. Women had taken more daily steps and aerobic steps compared to men, whereas men had higher bone loading indices at the radius and tibia (p-values \leq 0.003). These indices are described in more detail in the later chapter 4.2.7. Fractures were more common in men (464 vs. 581, p-value <0.001) but women sustained a greater number of forearm and wrist fractures. Women also reported a higher rate of eating disorder anorexia nervosa and use of corticosteroid medication compared to men (Table 2, p-values <0.02).

The number of treatment periods or hospital visits and diagnoses of eating disorders (anorexia nervosa and bulimia nervosa), epilepsy, Crohn's disease and ulcerative colitis from 1969 to 2014 in the Care Register for Health Care maintained by the National Institute for Health and Welfare were searched for according to the International Classification of Diseases 9th and 10th revisions (ICD-9 and ICD-10). In the group of eating disorders, there were 8 treatment periods or hospital visits in 3 study subjects from the present population. The diagnosis of epilepsy was found in 28 subjects who had 99 treatment periods or hospital visits. The corresponding numbers for Crohn's

disease were 64 treatment periods or hospital visits and 8 subjects with Crohn's disease and for ulcerative colitis 58 treatment periods or hospital visits and 21 subjects with ulcerative colitis.

4.2 Methods

4.2.1 Bone data

In 2008, subjects in the register of the Young Finns cohort received an invitation to the peripheral quantitative computed tomography (pQCT) and quantitative ultrasound (QUS) bone measurements (Laaksonen et al. 2010). A total of 1884 subjects (1059 women and 825 men) from Turku, Helsinki, Tampere, Oulu and Kuopio participated in the pQCT measurements. The corresponding number of the QUS measurements was 1953 (1094 women and 859 men).

Two functionally different bones, non-weight-bearing radius and weight-bearing tibia, were measured with the pQCT measurement device (XCT 2000R, Stratec Medizintechnik GmbH, Pforzheim, Germany). The same pQCT device was used in each study centre. Pregnant women were excluded from the measurements. For most of the cases, radius was measured on the non-writing hand and the tibia was measured on the left leg. Subjects with subdermal metallic objects or previous fractures within the scan area were measured from the contralateral side. The lengths of ulna and tibia were measured with a tape measure and the measurement lines of distal radius and tibia were defined as 4% and 5% from the cortical endplate, respectively. The diaphyseal sites were 30% for both studied bones. Altogether, 1842-1856 radius and 1853-1857 tibia measurements were successfully measured and analysed (~98% of those who participated).

Using the QUS technique (Sahara Clinical Bone Sonometer, Hologic Inc., Waltham, MA, USA) the speed of sound (SOS, m/s) and broadband ultrasound attenuation (BUA, dB/MHz) were measured primarily from the left heel. 1494-1515 ultrasound scans were successfully conducted and analysed (~77% of the participants).

The in vivo precision of the pQCT and QUS measurements was assessed through repeated scans of 39 volunteers (aged 24-64 years). Either radius or tibia or both bones and calcaneus were measured twice for each volunteer. The coefficients of variation (CV, %) for basic traits of radius and tibia varied between 0.5-4.4%. The CV values for SOS and BUA were 0.3% and 4.8%, respectively. The precision of pQCT scan analyses was tested using randomly selected scans of 157 subjects and no significant differences were found between the scan analyses. Additionally, the calibrations of pQCT and SAHARA devices were run with the daily phantom measurements.

This study was conducted according to the guidelines laid out in the World Medical Association Declaration of Helsinki – Ethical Principles for Medical Research Involving Human Subjects, and was approved by the local ethics committees of the participating universities. Written informed consent was obtained from all participants.

Table 2. Background characteristics of the study subjects in 2007.

	Women		Men		T-test/ χ^2 -test p-value
	N, total	Mean (SD) or frequency, n, (%)	N, total	Mean (SD) or frequency, n, (%)	
Age, years	1229	37.7 (5.0)	1002	37.7 (5.1)	0.72
Height, cm	1186	165.9 (6.0)	990	179.6 (6.6)	<0.001
Weight, kg	1185	69.9 (14.7)	987	86.4 (15.5)	<0.001
Body mass index (BMI), kg/m ²	1183	25.4 (5.1)	987	26.8 (4.2)	<0.001
Those measured as underweight, BMI <19, %	1183	34 (2.9%)	987	10 (1%)	0.002
Those measured as overweight, BMI >30, %	1183	193 (16.3%)	987	182 (18.4%)	0.19
Energy intake, kcal/day	1116	2178 (672)	880	2697 (947)	<0.001
Protein intake, g/day	1116	94.4 (31.2)	880	117.3 (39.9)	<0.001
Protein, E%	1116	17.4% (2.4)	880	17.6% (2.5)	0.11
Dietary calcium intake, mg/day	1116	1366 (547)	880	1546 (660)	<0.001
Dietary Vitamin D intake, μ g/day	1116	6.8 (2.9)	880	9.0 (3.9)	<0.001
Serum 25-OH vitamin D, nmol/l	1210	60.9 (20.5)	994	56.7 (16.9)	<0.001
Alcohol consumption, drinks/day	1212	0.6 (0.7)	993	1.4 (1.8)	<0.001
Excess alcohol intake (\geq 3 drinks/day), %	1212	19 (1.6%)	993	120 (12.1%)	<0.001
History of smoking (\geq 1 year), %	1184	442 (37.3%)	987	498 (50.5%)	<0.001
Current daily smokers, %	1225	183 (14.9%)	999	228 (22.8%)	<0.001
Steps/day	1070	7827 (2931)	806	7063 (2822)	<0.001
Aerobic steps/day	1070	2317 (2140)	802	1403 (1803)	<0.001
Bone loading index of radius	1004	344.6 (344)	770	431.9 (495)	<0.001
The lowest quarter of radial index (\sim low physical activity)	251	\leq 110.6	193	\leq 102.0	-
Bone loading index of tibia	1004	558.8 (571)	770	655.7 (742)	0.003
The lowest quarter of tibial index (\sim low physical activity)	251	\leq 173.6	193	\leq 140.0	-
Number of previous fractures	1005	464	768	581	<0.001 ^a
forearm or wrist fractures, %		123 (26.5%)		101 (17.4%)	
lower leg or ankle fractures, %		66 (14.2%)		82 (14.1%)	
Number of previous low-energy fractures	976	100	734	76	0.81 ^a
Parental low-energy fractures (% of all subjects)	1084	159 (14.7%)	814	92 (11.3%)	0.12
Epilepsy, %	1086	9 (0.8%)	819	9 (1.1%)	0.55
Crohn's disease or ulcerative colitis, %	1087	13 (1.2%)	819	7 (0.9%)	0.47
Anorexia nervosa, %	1087	9 (0.8%)	819	0	0.013 ^a
Hypogonadism (ovarial or testicular insufficiency), %	1082	5 (0.5%)	811	3 (0.4%)	-
Oral corticosteroid treatment (\geq 1 month), %	1087	73 (6.7%)	817	23 (2.8%)	<0.001

^ap-value from the Exact-test

4.2.2 Assessment of bone density and geometrical parameters

The analysis of bone density and geometrical traits from the pQCT scan images was done using specific threshold values and mode options (Stratec Medizintechnik GmbH). To define the outer contour of the bone and the total bone area, the counter mode 2 was used in the measurement analyses. Briefly, the counter mode 2 is an iterative contour detection procedure which begins by finding the first voxel of the outer bone edge. This voxel is then compared to a set of its neighbouring voxels and this process continues all around the bone, returning to the starting point. The outer threshold value for the separation of bone tissue from the surrounding soft tissue was 169 mg/cm^3 . The trabecular and cortical bone areas were then separated with a peel mode 2 in which an inner threshold of 480 mg/cm^3 was used at the distal bone sites and 710 mg/cm^3 at the bone shafts. Filtration of bone area with threshold algorithm ignores isolated high attenuation voxels in the trabecular areas and in areas that are not continuous. Analyses of the computed tomography measurements yielded the following bone parameters: bone mineral content (BMC, mg), trabecular and cortical bone mineral densities (mg/cm^3), and total and cortical bone areas (mm^2). Additionally, three bone strength indices were calculated: stress-strain index (SSI, mm^3), bone strength index (BSI, g^2/cm^4) and cortical strength index (CSI). SSI predicts the torsional bone strength which is based on the calculation of the cross-sectional moment of inertia divided by the maximum distance of any voxel from the centre of gravity. To take the material properties into consideration, the SSI formula also contains a quotient of calculated cortical density and maximal physiological cortical density. BSI that represents the compressive bone strength was calculated as a product of squared total bone mineral density and total cross-sectional area (total density² x total area) (Kontulainen et al. 2002). The value of CSI was received from the ratio of cortical bone area and total bone area (cortical area/total area) (Nikander et al. 2009).

4.2.3 Assessment of fractures

Information on all fractures was collected with a questionnaire in 2008. Bone fracture type, how and when the fracture occurred and the site of the fracture were reported separately. Fractures were classified as low-energy fractures if sustained as a result of a fall from no more than standing height. Fractures caused by a fall from greater heights, sport injuries involving other people, collisions or accidents involving vehicles or high velocities such as cycling, skiing, skating or motorised vehicles were excluded from the low-energy fracture category.

4.2.4 Genotyping

Genetic analysis of the lactase gene polymorphism (C/T-13910) was done from blood samples collected in 2001. Genomic DNA was extracted from peripheral blood leucocytes using a commercial kit (Qiagen, Hilden, Germany). Lactase C/T-13910 genotyping (*rs4988235*) was performed using a 5' nuclease assay and fluorogenic, allele-specific TaqMan probes and primers with the ABI Prism 7000 Sequence Detection System (Applied Biosystems, Foster City, CA, USA).

The APOE genotypes were analysed with SNPs *rs429358* and *rs7412*, and the APOE promoter polymorphisms -219 and +113 with *rs405509* and *rs440446*, respectively. DNA was extracted from peripheral blood leukocytes by using the QIAamp®DNA Blood Minikit and automated biorobot M48 extraction (Qiagen, Hilden, Germany). Genotyping was performed by using Taqman®SNP Genotyping Assays (*rs429358* assay C 3084793_20; *rs7412* assay C_904973_10; *rs405509* assay C_905013_10; *rs440446* assay C_905012_20) and the ABI Prism 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA, USA).

No discrepancies emerged in the genotyping of duplicate samples of these polymorphisms.

4.2.5 Clinical factors

Body weight and height were measured, and body mass index (BMI, kg/m²) was calculated using standard methods. Alcohol consumption was estimated from the food frequency questionnaire (FFQ) as an energy percent of total energy intake (E%) and from the self-administered questionnaire as standard number of drinks per day. Use of oral corticosteroid medication for at least one month (yes/no) and smoking habits were also collected with the questionnaires. Smoking was defined as pack-years (the number of years a person has smoked one pack of cigarettes per day) and as a portion of those who smoke at least once a week (%) or daily (%). Pubertal status of children and adolescents in 1980 was examined and classified according to the Tanner scale (1-5). Females were also asked about their menarche age (years), parity and duration of lactation (in months) with a self-administered questionnaire.

Subjects' venous blood samples were drawn after an overnight fast and serum was separated for the biochemical analysis (Raiko et al. 2010). The levels of serum total cholesterol, high-density lipoprotein (HDL)-cholesterol and glucose were measured using enzymatic assays performed on an AU400-analyser (Olympus, Japan). Low-density lipoprotein (LDL)-cholesterol was estimated using the Friedewald formula in subjects with triglycerides <4.0 mmol L⁻¹. All the biochemical analyses were carried out in the Laboratory for Population Research of the National Institute for Health and Welfare (Turku, Finland). Additionally, due to changes in methods or kits between the years 2001 and 2007, the levels of glucose and insulin were corrected using correction factor equations (Raiko et al. 2010). Serum calcidiol concentrations (in nmol/l) were determined using radioimmunoassay (DiaSorin, Stillwater, Minnesota).

Maximal oxygen consumption (VO₂max) and work rate (WR_{max}) were obtained from the exercise tests performed on electronically braked cycle ergometers in a subpopulation of the Young Finns Cohort during the years 2007-2009 (n=538) (Lode Corival 906900, Lode BV, Groningen, Netherlands). During the tests, electrocardiography was recorded (Corina ECG amplifier and CardioSoft acquisition software ver. 4.2, GE Medical Systems, Freiburg, Germany) and breath-by-breath measurements were performed with ventilator gas analysers (V-max 29C, SensorMedics, Yorba Linda, CA, USA and Jaeger Oxycon Pro, VIASYS Healthcare GmbH, Hoechberg, Germany). VO₂max was determined as the highest oxygen uptake during the last 30-second averaged interval

and WRmax was calculated by adding the work done during the last full minute interval to the fraction of the work performed over the final, interrupted interval (Hulkkonen et al. 2014).

4.2.6 Assessment of dietary factors

At the baseline in 1980, dietary data was gathered with 48-hour dietary recall interviews done by trained dietitians for half of the study subjects (Räsänen et al. 1985). The interviews were repeated in 1986 and 2001 with participation rates of 59-68% (Mikkilä et al. 2005). This method gives detailed information on subjects' dietary habits during the two days prior to the interview. In 2007, information on food consumption and nutrient intake was collected with a modified 131-item FFQ developed by the Finnish National Institute for Health and Welfare (Paalanen et al. 2006). In the beginning, the nutrient calculations were based on the Finnish food composition tables maintained by the University of Helsinki and analytical data was obtained from the local food industry. Later in 2001 and 2007, the nutrient contents of reported foods were calculated using the Finnish Food Composition database, Fineli® (National Institute for Health and Welfare).

The calcium index consisted of foods with high calcium content and foods that were regularly consumed together with milk (Study I). In the index, one unit of calcium was equivalent to the amount of calcium in one glass of milk (180 ml containing 217 mg calcium). Food items that were included in the calcium index were breakfast cereal, cheese, chocolate, coffee, fish, fresh fruits and vegetables, ice cream, juice, mineral water, milk, sour milk and yogurt, which together composed 90.5% of the total calcium intake in the present population. After this, calcium index was energy adjusted using the residual method (Willett 1998) and was divided into tertiles (<4.69, 4.70-6.81, >6.82). In Study II, the intake of saturated fat (SAFA, g/day) in 1980, 1986, 2001 and 2007 was combined as the mean energy-adjusted longitudinal intake of SAFA, and then grouped into tertiles for further analysis.

In addition, intakes of energy (kcal/day), protein (E% and g/day), calcium (mg/day) and vitamin D (µg/day) were presented as a mean intake/day or as an energy percentage of total energy intake (Studies I-IV). Intake of milk products was given as g/1000 kcal.

4.2.7 Measures of physical activity

Four different measures of physical activity were used in the present thesis: radial and tibial bone loading indices (Study I), physical activity index (PAI) (Study III) and pedometer-determined steps (Study IV). Bone loading indices were collected through the questions according to the type of mechanical loading exerted on bone: high impacts (ball games, aerobics, track and field events etc.), strength training (gym and circuit training etc.), repetitions (walking, running, skiing etc.), non-weight bearing exercise (cycling, swimming etc.) and work or recreational activities (working in the garden, construction work etc.). Different coefficients were used to weight the effects of loads exerted on bones (Laaksonen et al. 2010). Bone loading indices were separately calculated with the following equation for each type of mechanical loading: weight of the subject (kg) x EL x I

$x \ln(1+F \times D)$, where EL=exercise loading, I=intensity of exercise, F=frequency of exercise and D=duration of exercise. After this, indices for different types of mechanical loading were combined to form the total bone loading indices for radius and tibia (Study I).

In Study III, the sum indices of physical activity collected with the questionnaires in 1980 were used to describe habitual physical activity and inactivity in childhood and adolescence (Telama et al. 2005, Telama et al. 2014). The questions regarding physical activity of 3 to 6-year-olds were addressed to their mothers and directly to the 9 to 18-year-old participants. For 3-6 year-old children, the PAI included hours spent playing outdoors, children's physical activity compared to other children, and children's intensity and enjoyment during playing. Mothers were also asked whether their child had been instructed to do activities or sports and what their child usually did outdoors. By summing these variables together, the PAI of 3-6-year-olds was formed with scores ranging from 9 to 23 (9 indicating low activity). For older children and adolescents, the PAI consisted of intensity (breathlessness and sweating during exercise), leisure-time physical activity (frequency of exercise lasting half an hour or more per session), participation in sport club training (how often) and competitions (yes/no), and common activities in leisure-time. Intensity and frequency were coded as 1 indicating inactivity or very low activity level, 2=moderate level, and 3=frequent or vigorous physical activity. By summing the variables, the PAI of 9-18-year-old children and adolescents was formed with scores from 5 to 14 (5 indicating low activity). In 2007, PAI included items on intensity of physical activity, frequency and duration of vigorous physical activity, average duration of a physical activity sessions, and participation in organised exercise.

In Study IV, steps were collected from 1874 individuals (Hirvensalo et al. 2011) using Omron Walking Style One pedometers (HJ-152R-E, Omron Healthcare Co., LTD. Kyoto, Japan). Pedometers were required to be attached on the waistband or belt in the same position for 7 consecutive days and to be removed while bathing or doing other water activities. At the end of each day, the number of total steps was recorded in pedometer logs. Participants who had at least 4 monitoring days with at least 8 hours pedometer wear time were included in all the analyses (n=1853). Sickness or injury events, exceptional step count recorded as an atypical day, or problems with pedometer use, were replaced by the mean of other days. The used Walking Style One pedometer measured total steps over a distance of 1000 meters with 0.1-1.3% accuracy in different walking situations (Hirvensalo et al. 2011) and the correlation coefficient with steps measured by the accelerometer (GT1M, ActiGraph, Pensacola, FL, USA) was 0.966 ($p < 0.001$) (Mansikkaniemi et al. 2012).

4.2.8 Statistical methods

Distributions of bone parameters were studied using nonparametric tests and figures. The Hardy-Weinberg equilibrium of the gene polymorphisms was performed using an exact test of the Genepop software (Rousset 2008). In Study II, subjects were divided into the APOE $\epsilon 4$ allele carriers ($\epsilon 3/\epsilon 4$ and $\epsilon 4/\epsilon 4$) and the non-carriers ($\epsilon 2/\epsilon 2$, $\epsilon 2/\epsilon 3$ and $\epsilon 3/\epsilon 3$) prior to the statistical analyses. The $\epsilon 4/\epsilon 2$ genotype (n=34) was excluded from the analyses since it was difficult to design in a group ($\epsilon 2$ and $\epsilon 4$ alleles usually have opposite effects). The analyses of APOE -219 and

+113 promoter polymorphisms were performed within the most common APOE $\epsilon 3/\epsilon 3$ genotype to exclude the confounding effect of the APOE $\epsilon 2/\epsilon 3/\epsilon 4$ genetic variation. In Study III, the childhood (3-6 years) and adolescence (9-18 years) physical activity sum indices were divided into three or four groups according to the number of participants in each physical activity score level. The cut-off values of childhood PAI were <15 and <17 in females and <16 and <18 in males. The corresponding values for adolescence PAI were <8, <9 and <10 in females and <9, <10 and <11 in males. According to these classifications three childhood physical activity groups were named: low, moderate and frequent. Respectively, the four adolescent physical activity groups were: very low, low, moderate frequent. In Study IV, the cut-off values for the tertiles of total steps were < 6317 and > 8765 in women and < 5514 and > 7962 in men.

Levene's test was used to test the homogeneity of group variances before the analysis of variance (ANOVA), Welch-test and Brown-Forsythe-test which were all used in addition to a Chi-square test to compare the subject characteristics. The dose-dependent between-group differences in pQCT and QUS bone traits were examined through covariance analysis (ANCOVA) using selected variables as covariates. Additionally, gene-diet interactions with pQCT bone variables were examined using ANCOVA (Studies I and II) and the linear trends using polynomial contrast (Studies II and IV). In Study I, a Chi-square test was also used to compare the frequencies of low-energy fractures across the groups of lactase gene polymorphism and calcium index. In Study III, the adjusted odds ratios (ORs) of tibial pQCT bone traits and low-energy fractures in different physical activity groups (the lowest activity group and those without fractures as the references) were calculated with Binary Logistic regression analysis. All comparisons were done separately for women and men because of different endocrine profiles. Statistical analyses were carried out with SPSS 16 software, PASW Statistics 17, IBM SPSS Statistics 22 and 24 (Studies I-IV). P-values ≤ 0.05 were considered statistically significant.

5. RESULTS

5.1 Lactase gene C/T-13910 polymorphism, dietary calcium and pQCT bone traits of radius and tibia

The frequencies of T/T, T/C and C/C (-13910) genotypes were 35.4% (n=312), 48.3% (n=426) and 16.3% (n=144) in women and 35.7% (n=239), 48.7% (n=326) and 15.5% (n=104) in men, respectively (Study I). Distributions of lactase genotypes followed the Hardy-Weinberg distributions (H-W exact test, p-value 0.97). The mean intake of calcium was lowest in men with the C/C-13910 genotype (p-value 0.001). The difference between the carriers of T/T and C/C was on average 282 mg/day. In women, no differences were found in radial or tibial pQCT bone traits across the lactase genotypes (Study I). In men, small differences were seen in trabecular densities at the distal radius and tibia, which were ~ 3% higher in men with the T/T-13910 genotype compared to the other lactase genotypes (p-values 0.03 and 0.02). The studied interactions of lactase polymorphism with calcium index and bone loading indices on bone phenotypes were not statistically significant in the present population. In addition, no differences were found in the frequency of low-energy fractures between the C/T-13910 genotypes.

Background characteristics and pQCT bone traits were also compared between the tertiles of calcium index (Study I). Both women and men in the highest tertile of calcium index had the lowest alcohol consumption and in the middle tertile the lowest energy intake on average (p-values ≤ 0.002). Men in the highest tertile of calcium index also had the youngest mean age (p-value 0.001). In men, variation was found in radial and tibial bone phenotypes in the groups of calcium index. Trabecular density at the distal radius, CSI at the radial shaft, BMC, trabecular density, CSI and BSI at the distal tibia, and BMC and CSI at the tibial shaft were greater in men in the two upper tertiles of calcium index than in the lowest calcium group (p-values < 0.005). Differences were also seen in the frequencies of low-energy fractures since 17.2%, 13.4% and 9.2% of men had low-energy fractures in the lowest, second and third tertiles of calcium index, respectively (chi-square test, p-value 0.05).

5.2 Apolipoprotein E gene polymorphisms, longitudinal saturated fat intake and pQCT bone traits of radius and tibia

5.2.1 APOE gene polymorphism

The frequencies of six APOE genotypes ($\epsilon 2/\epsilon 2$, $\epsilon 3/\epsilon 2$, $\epsilon 4/\epsilon 2$, $\epsilon 3/\epsilon 3$, $\epsilon 4/\epsilon 3$ and $\epsilon 4/\epsilon 4$) are shown in Table 3. The APOE genotype distribution followed the Hardy-Weinberg distribution (H-W exact test, p-value 0.89). On average, the carriers of APOE $\epsilon 4$ allele had 5-5.5% higher total cholesterol and 8.4-9.5% higher LDL-cholesterol serum levels compared to those without the $\epsilon 4$ allele (ANOVA, p-values ≤ 0.001) (Study II). In men, the $\epsilon 4$ allele carriers also had 0.05 mmol/l lower mean HDL-cholesterol levels than the non-carriers (p-value 0.05).

In terms of radius, no differences were found between the carriers and non-carriers of the APOE $\epsilon 4$ allele. At the tibial diaphysis, women with the $\epsilon 4$ allele had somewhat lower cortical density than the non-carriers (ANCOVA, p-value 0.02) (Study II). However, in men, BMC at the tibial shaft was greater in the $\epsilon 4$ allele carriers than the non-carriers (ANCOVA, p-value 0.05). In addition, no interactions of the APOE $\epsilon 2/\epsilon 3/\epsilon 4$ genetic variation with saturated fat intake on bone traits at the radius and tibia were found in the present population.

5.2.2 APOE -219G/T and +113G/C promoter polymorphisms

The frequencies of the -219G/T and +113G/C genotypes (G/G, G/T, T/T and G/G, G/C, C/C) within the APOE $\epsilon 3/\epsilon 3$ genotype are shown in Table 3. These two polymorphisms were in high linkage disequilibrium ($r^2=0.98$, $D'=0.99$) and therefore all the results are presented for the -219G/T genotypes (G/G, G/T and T/T) only, meaning that almost identical results were found in women and men with the +113G/C genotypes (G/G, G/C and C/C) (Study II). The APOE promoter -219G/T genotype distribution followed the Hardy-Weinberg distribution (H-W exact test, p-value 0.53). A few differences were noticed in the characteristics between the -219G/T genotypes as women with the T/T allele had the lowest serum glucose levels (ANOVA, p-value 0.03) and men with the T/T allele the lowest alcohol consumption (Welch-test, p-value <0.001).

Table 3. Frequencies of the APOE genotypes ($\epsilon 2/\epsilon 2$, $\epsilon 3/\epsilon 2$, $\epsilon 4/\epsilon 2$, $\epsilon 3/\epsilon 3$, $\epsilon 4/\epsilon 3$ and $\epsilon 4/\epsilon 4$) and the APOE -219 and +113 promoter genotypes (G/G, G/T, T/T and G/G, G/C, C/C) within the APOE $\epsilon 3/\epsilon 3$ genotype presented in women and men.

Genotypes	$\epsilon 2/\epsilon 2$: rs429358 (T/T) and rs7412 (T/T)	$\epsilon 3/\epsilon 2$: T/T and C/T	$\epsilon 4/\epsilon 2$: T/C and C/T	$\epsilon 3/\epsilon 3$: T/T and C/C	$\epsilon 4/\epsilon 3$: T/C and C/C	$\epsilon 4/\epsilon 4$: C/C and C/C
Women	2 (0.2%)	74 (7.3%)	23 (2.3%)	567 (56%)	304 (30%)	42 (4.2%)
Men	2 (0.3%)	43 (5.4%)	11 (1.4%)	471 (58.9%)	244 (30.5%)	28 (3.5%)
Genotypes	-219G/G	-219G/T	-219T/T	+113G/G	+113G/C	+113C/C
Women	214 (38.2%)	268 (47.9%)	78 (13.9%)	211 (38.4%)	263 (47.8%)	76 (13.8%)
Men	187 (40.2%)	220 (47.3%)	58 (12.5%)	193 (41.7%)	212 (45.8%)	58 (12.5%)

At the distal radius, women with the -219T/T allele had the lowest cortical and compressive strength indices (linear trend, p-values ≤ 0.05) (Study II). The mean values of CSI and BSI were respectively 8.3% and 7.4% lower than in women with the -219G/G allele. In contrast, men with the -219T/T allele had the largest total areas at the distal and shaft sites of radius, and the highest SSI at the radial shaft (linear trend, p-values ≤ 0.05). Compared to the -219G/G male carriers, values were 4-5.1% greater in men with the T/T allele. Men with the -219T/T allele had, however,

also the lowest cortical density and CSI at the radial shaft (the mean difference 0.8-2.2% compared to the G/G genotype, linear trend, p -values ≤ 0.05).

At the distal tibia, women with the -219T/T allele had the lowest values of CSI and BSI, which were on average 7.8% and 6.7% lower compared to women with the -219G/G genotype (linear trend, p -values ≤ 0.03) (Study II). At the tibial shaft, the mean values of CSI were 1.8% and 2.5% lower in women and men with the T/T allele than the G/G carriers, respectively (linear trend, p -values ≤ 0.04).

The interactions of saturated fat with the APOE -219G/T genotypes on radial and tibial bone traits were studied and found only in women at the radial shaft (Figure 3). The mean total area and SSI at the radial shaft were the lowest in women with the -219T/T allele, whose saturated fat intake was the highest (p -values for interaction ≤ 0.02).

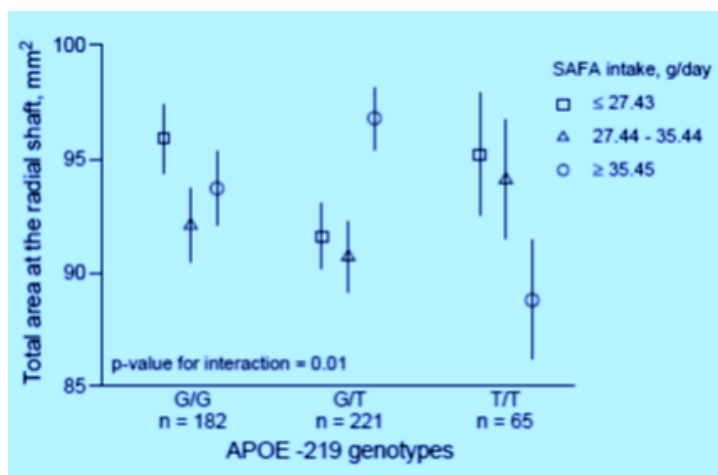


Figure 3. Interaction of the APOE -219 promoter polymorphism with saturated fat intake on total area at the radial shaft in women. Values are expressed as means, and error bars as standard errors of means.

5.3 Physical activity in adolescence, pQCT bone traits of tibia and risk of low-energy fractures

There were some differences in the characteristics of adolescent physical activity groups in both genders (Study III). Females aged 9-18-years with moderate or frequent physical activity levels were younger and smaller in body size than females in the two lowest activity levels (p -values < 0.001 for group differences). The use of milk products was the greatest in females with the most frequent activity level (p -value 0.002) and also mean serum calcidiol levels tended to be greater in more active females (p -value < 0.001). In adulthood, females with higher activity levels were still more active than their less active peers (p -value 0.001). They also drank less alcohol than their peers (p -value 0.01). Males aged 9-18-years with a moderate activity level had the youngest mean age and smallest body size on average (p -values < 0.001). Serum calcidiol levels were greatest in

males with frequent activity levels for both measurement points (p-value ≤ 0.04). In 2007, age and height differed statistically significantly across the male adolescent physical activity groups (p-values ≤ 0.02). Those with a moderate activity level were still the youngest but height was the lowest on average in males with the lowest activity level. As in females, adult physical activity level was the greatest in males with the highest adolescent activity level (p-value 0.04).

During the follow-up, 100 females and 76 males sustained low-energy fractures (Table 2, page 23). As shown in Tables 4 and 5, the risk of low-energy fractures did not differ significantly between the physical activity groups of children and adolescents.

Table 4. The adjusted odds ratios¹ (ORs) of low-energy fractures (95% confidence intervals) in the physical activity groups of 3-6-year-olds.

	Childhood physical activity			P-value for trend
	Low (reference)	Moderate	Frequent	
Females				
n=225	n=57	n=76	n=92	0.31
ORs	1	0.53 (0.13-2.23)	1.54 (0.45-5.21)	
Males				
n=196	n=62	n=67	n=67	0.27
ORs	1	1.69 (0.52-5.49)	2.09 (0.57-7.67)	

¹Covariates used in the model: age, weight, height, adult smoking, adult alcohol consumption, parental low-energy fractures, corticosteroid medication, serum calcidiol levels, adult physical activity and in females also parity and lactation.

Table 5. The adjusted odds ratios¹ (ORs) of low-energy fractures (95% confidence intervals) in the physical activity groups of 9-18-year-olds.

	Adolescent physical activity				P-value for trend
	Very low (reference)	Low	Moderate	Frequent	
Females					
n=489	n=119	n=137	n=108	n=125	0.86
ORs	1	1.79 (0.79-4.05)	1.05 (0.42-2.64)	1.15 (0.47-2.83)	
Males					
n=476	n=159	n=94	n=100	n=123	0.28
ORs	1	1.03 (0.48-2.22)	0.91 (0.42-2.00)	0.62 (0.27-1.43)	

¹Covariates used in the model: age, weight, height, adult smoking, adult alcohol consumption, parental low-energy fractures, corticosteroid medication, serum calcidiol levels, adult physical activity and in females also parity and lactation.

The adjusted odds ratios (ORs) of adult tibial pQCT bone traits in the adolescent physical activity groups are shown in Tables 6 and 7. In females, frequent physical activity at the age of 9-18 years

was associated with less likely below median values of BSI at the distal tibia, and total and cortical areas, BMC, CSI and SSI at the tibial shaft compared to females with very low activity level in adolescence (ORs 0.33-0.53, $P \leq 0.05$; P-values for trend 0.002-0.05, Table 6). Cortical density at the tibial shaft showed the opposite trend (P-value for trend 0.03, Table 6). In males, total area at the distal tibia, and cortical area and CSI at the tibial shaft were less likely to be under the median values in those who exercised frequently in adolescence compared to males with the lowest activity level (ORs 0.48-0.53, $P \leq 0.05$; P-values for trend 0.01-0.02, Table 7). According to the childhood physical activity of 3-6-year-old children, no significant differences were found in adult tibial bone traits (Study III).

Table 6. The adjusted odds ratios¹ (ORs) of adult tibial pQCT bone traits (95% confidence intervals), testing the likelihood of females having below median values when grouped by their physical activity at the age of 9-18 years.

pQCT bone traits ² with median values	Adolescent physical activity in females				P-value for trend
	Very low (reference)	Low OR (95% CI)	Moderate OR (95% CI)	Frequent OR (95% CI)	
<i>Distal tibia (5%)</i>					
Total area, mm ² (809.7)	n=116 1	n=128 1.20 (0.67-2.14)	n=104 0.81 (0.44-1.51)	n=124 0.73 (0.39-1.34)	0.19
BMC, mg (516.2)	1	1.05 (0.59-1.89)	0.93 (0.49-1.76)	0.58 (0.31-1.10)	0.08
Trabecular density, mg/cm ³ (226.7)	1	0.73 (0.42-1.26)	1.11 (0.62-2.01)	0.77 (0.43-1.39)	0.66
BSI, g ² /cm ⁴ (0.817)	1	0.75 (0.43-1.31)	0.88 (0.48-1.62)	0.44 (0.24-0.81)	0.02
<i>Tibial shaft (30%)</i>					
Total area, mm ² (339.0)	n=116 1	n=127 0.95 (0.51-1.77)	n=104 0.75 (0.38-1.46)	n=123 0.54 (0.28-1.05)	0.05
Cortical area, mm ² (244.5)	1	0.70 (0.38-1.28)	0.80 (0.42-1.54)	0.33 (0.17-0.64)	0.002
Cortical density, mg/cm ³ (1170)	1	1.41 (0.80-2.46)	1.48 (0.81-2.72)	1.99 (1.09-3.63)	0.03
BMC, mg (571.9)	1	0.92 (0.50-1.69)	0.93 (0.49-1.79)	0.38 (0.20-0.73)	0.01
CSI (0.720)	1	0.65 (0.38-1.11)	0.85 (0.47-1.51)	0.53 (0.29-0.96)	0.08
SSI, mm ³ (1282)	1	0.94 (0.51-1.74)	0.89 (0.46-1.73)	0.52 (0.27-0.99)	0.05

¹Covariates used in the model: age, weight, height, adult smoking, adult alcohol consumption, parental low-energy fractures, corticosteroid medication, serum calcidiol levels, adult physical activity, pubertal status, menarche age, parity and lactation.

²BMC, bone mineral content; CSI cortical strength index; BSI, bone strength index; SSI, stress-strain index.

Table 7. The adjusted odds ratios¹ (ORs) of adult tibial pQCT bone traits (95% confidence intervals), testing the likelihood of males having below median values when grouped by their physical activity at the age of 9-18 years.

pQCT bone traits ² with median values	Adolescent physical activity in males				P-value for trend
	Very low (reference)	Low OR (95% CI)	Moderate OR (95% CI)	Frequent OR (95% CI)	
<i>Distal tibia (5%)</i>	n=150	n=91	n=94	n=120	
Total area, mm ² (983.4)	1	0.85 (0.46-1.56)	0.60 (0.31-1.17)	0.51 (0.27-0.92)	0.02
BMC, mg (694.8)	1	0.70 (0.38-1.27)	1.06 (0.57-1.99)	0.69 (0.39-1.24)	0.38
Trabecular density, mg/cm ³ (256.2)	1	0.85 (0.49-1.47)	1.12 (0.63-1.97)	1.13 (0.66-1.92)	0.53
BSI, g ² /cm ⁴ (1.244)	1	0.74 (0.42-1.31)	0.94 (0.52-1.69)	0.79 (0.45-1.37)	0.52
<i>Tibial shaft (30%)</i>	n=148	n=91	n=94	n=120	
Total area, mm ² (431.9)	1	1.31 (0.69-2.48)	0.65 (0.33-1.29)	0.62 (0.33-1.15)	0.06
Cortical area, mm ² (317.0)	1	0.85 (0.45-1.62)	0.66 (0.34-1.31)	0.48 (0.25-0.90)	0.02
Cortical density, mg/cm ³ (1148)	1	1.04 (0.59-1.83)	1.18 (0.66-2.12)	1.23 (0.71-2.14)	0.42
BMC, mg (725.9)	1	1.05 (0.56-1.96)	0.75 (0.39-1.45)	0.64 (0.35-1.18)	0.11
CSI (0.740)	1	0.60 (0.34-1.03)	0.53 (0.30-0.94)	0.50 (0.29-0.85)	0.01
SSI, mm ³ (1793)	1	1.01 (0.53-1.90)	0.72 (0.36-1.43)	0.69 (0.37-1.29)	0.19

¹Covariates used in the model: age, weight, height, adult smoking, adult alcohol consumption, parental low-energy fractures, corticosteroid medication, serum calcidiol levels, adult physical activity and pubertal status.

²BMC, bone mineral content; CSI cortical strength index; BSI, bone strength index; SSI, stress-strain index.

5.4 Daily steps, calcaneal bone traits and pQCT bone traits of tibia and radius

In Study IV, women and men in the highest tertile of daily steps had lower body weight and BMI than their less active study peers (p-values for group differences ≤ 0.003). They also smoked less and had higher physical activity score and maximal work output in a cycle ergometer exercise test compared to their counterparts (p-values ≤ 0.05). In addition, women within the highest tertile of daily steps had the highest mean serum calcidiol level whereas in men, the highest mean calcidiol level was found in the middle tertile of steps (p-values < 0.001). In men, the maximal oxygen consumption reflecting physical endurance capacity was also the highest among men in the highest tertile of daily steps (p-value 0.001).

The differences in BUA and SOS values at the calcaneus were 3.8% and 0.5% greater in women in the highest tertile of daily steps compared to the lowest tertile (Table 8, p-values for trend ≤ 0.04). Similarly in distal tibia, women taking over 8765 steps/day had 1.1% larger total cross-sectional area, 3.2% higher BMC, 3.7% denser trabeculae and 5.4% greater BSI than women in the lowest tertile of daily steps (Table 8, p-values for trend ≤ 0.02). In tibial shaft, bone area was 1.7%, cortical bone area 1.6%, BMC 1.7% and SSI 2.7% greater among women in the highest tertile of daily steps than the other women (Table 8, p-values for trend ≤ 0.02). In distal radius, women in the highest tertile of daily steps had 2.2% higher BMC and 3.4% greater BSI compared to the lowest tertile (Table 8, p-values for trend ≤ 0.04). In the radial shaft, total cross-sectional bone area was 2.3%, cortical area 1.7%, BMC 1.7% and SSI 2.4% greater in women in the highest tertile compared to the lowest or middle tertiles of daily steps (Table 8, p-values for trend ≤ 0.03).

In men, no statistically significant differences were found in calcaneus or radius between the tertiles of daily steps (Study IV). In tibia, total cross-sectional bone area and BMC at the distal site were the highest in men within the middle tertile of steps, whereas bone area and SSI at the tibial shaft were greatest in men in the lowest tertile of daily steps (p-values for trend ≤ 0.04).

Table 8. Unadjusted mean values (SD) of quantitative ultrasound (QUS) and peripheral quantitative computed tomography (pQCT) bone traits in women in the tertiles of daily steps.

Bone traits ^a	N=223-267	N=231-285	N=251-285	ANCOVA ^b	
	< 6317 steps/day	6317-8765 steps/day	> 8765 steps/day	P-value	P-value for trend
<i>At the calcaneus</i>					
BUA, dB/MHz	77.6 (14.3)	78.8 (15.6)	80.7 (16.6)	0.10	0.04
SOS, m/s	1555 (28.7)	1557 (27.9)	1563 (30.2)	0.02	0.01
<i>At the distal tibia (5% from the cortical endplate)</i>					
Total area, mm ²	809.8 (101.3)	815 (118)	818.7 (103.8)	0.04	0.02
BMC, mg	511.1 (69.4)	521.1 (71.7)	527.8 (70.6)	< 0.001	< 0.001
Trabecular density, mg/cm ³	224.2 (28.4)	229.6 (31.1)	232.7 (30.7)	0.001	< 0.001
BSI, g ² /cm ⁴	0.819 (0.189)	0.848 (0.196)	0.866 (0.208)	0.002	0.001
<i>At the tibial shaft (30%)</i>					
Total area, mm ²	341 (44.6)	338.9 (42.1)	344.9 (41.9)	0.001	0.01
Cortical area, mm ²	245.3 (30.7)	245.9 (30)	249.2 (30.3)	0.02	0.01
Cortical density, mg/cm ³	1168 (21.6)	1169 (17.5)	1170 (19.3)	0.90	0.65
BMC, mg	572.8 (69.2)	574.7 (68.7)	582.5 (68.3)	0.02	0.02
CSI	0.72 (0.05)	0.73 (0.05)	0.72 (0.05)	0.36	0.93
SSI, mm ³	1296 (241.9)	1287 (234.3)	1322 (225.3)	0.002	0.01
<i>At the distal radius (4%)</i>					
Total area, mm ²	310.9 (42.2)	312.2 (47.5)	314.7 (46.7)	0.25	0.12
BMC, mg	195.2 (28.9)	197.9 (28.4)	199.6 (30.6)	0.04	0.01
Trabecular density, mg/cm ³	205.8 (27.8)	206.8 (28.3)	208.5 (30.2)	0.48	0.22
BSI, g ² /cm ⁴	0.314 (0.09)	0.321 (0.08)	0.325 (0.09)	0.13	0.04
<i>At the radial shaft (30%)</i>					
Total area, mm ²	92.8 (13.2)	92.6 (14.2)	94.8 (13.8)	0.008	0.01
Cortical area, mm ²	74.4 (8.4)	74.7 (8.4)	75.7 (8.2)	0.01	0.006
Cortical density, mg/cm ³	1211 (19.5)	1213 (21)	1210 (20)	0.16	0.19
BMC, mg	180.1 (20.2)	181.2 (20.2)	183.2 (19.6)	0.03	0.01
CSI	0.81 (0.06)	0.81 (0.06)	0.81 (0.06)	0.12	0.50
SSI, mm ³	203.8 (38.4)	203.9 (42.1)	208.8 (39)	0.03	0.03

^aBUA, broadband ultrasound attenuation; SOS, speed of sound; BMC, bone mineral content; BSI, bone strength index; CSI, cortical strength index; SSI, stress-strain index.

^bCovariates used in the model: age, height, weight, serum calcidiol level and physical activity index.

6. DISCUSSION

6.1 The Young Finns Study and bone data

The present study population was drawn from a large, representative sample of Finnish women and men who were invited to the follow-up examinations seven times after the baseline study (Raitakari et al. 2008). The pQCT and QUS bone data was collected in 2008. A major advantage is that comprehensive information on many health and lifestyle factors affecting bone health was gathered during the study years and it was thus possible to consider these various factors when interpreting the results. In addition to the bone measurements, study subjects were asked about their previous and present fractures with a questionnaire; their answers were then further classified (or not) into low-energy fractures used in Studies I and III. Since fractures were not validated with any other method there might be some reporting errors due to recall bias.

The pQCT measurements from radius and tibia provide a reasonable assessment of cross-sectional area, trabecular and cortical densities and strength properties with a low radiation dose (Sievänen et al. 1998). Although other clinically important bones like lumbar spine or femoral neck were not evaluated in the present thesis, geometrical parameters of radius and tibia have earlier discriminated subjects with and without fractures, and associated well with the bone failure properties measured in the tibia (Vico et al. 2008, Kontulainen et al. 2008).

The difference in successful measurements between the pQCT and QUS techniques was around 21% in favour of the pQCT. In total, approximately 23% of the QUS measurements were unsuccessful due to failed propagation of ultrasound at the calcaneus, which was reported as excess noise, invalid measurement or measurement out of range. In vivo precision of the pQCT and QUS measurements assessed with the coefficient of variation was, however, rather low, varying between 0.3% and 4.8% (n=39). Personnel in each study centre were trained to perform the measurements with the same protocol. There were, however, differences between the study centres in the measurement frequencies, with Oulu having the lowest numbers, but this was partly due to subjects' relative lack of willingness to participate in the measurements.

6.2 Genetic perspective

6.2.1 Lactase gene C/T-13910 polymorphism

The main finding in Study I was that men with the T/T-13910 genotype had ~3% higher trabecular density at the distal radius and tibia compared to the other lactase genotypes. Other bone traits or low-energy fractures were not associated with the C/T-13910 polymorphism in the present population of women and men. In addition, trabecular density at the distal radius, CSI at the radial shaft, BMC, trabecular density, CSI and BSI at the distal tibia, and BMC and CSI at the tibial shaft were greater in men in the two upper tertiles of calcium index than in men who were in the lowest calcium tertile (p-values <0.005). In addition, the frequencies of low-energy fractures in men were

17.2%, 13.4% and 9.2% in the lowest, second and third tertiles of calcium index, respectively (chi-square test, p-value 0.05).

In previous studies including postmenopausal women and elderly people, the C/C-13910 carriers with adult-type hypolactasia had lower BMD and higher bone fracture incidence than subjects with the T/C and T/T (-13910) genotypes (Obermayer-Pietsch et al. 2004, Enattah et al. 2005, Bácsi et al. 2009). However, the present findings on this matter are contradictory since other studies in young adults (Enattah et al. 2004, Laaksonen et al. 2009) and postmenopausal women (Enattah et al. 2005) failed to confirm these results. Different study results may be due to the differences in study designs since elderly people are more prone to bone frailty than younger adults. In the present study, it was noticed that men with the T/T-13910 genotype had the highest mean calcium intake, which may have contributed to their trabecular densities at the distal sites of radius and tibia. However, all men genotyped for the C/T-13910 polymorphism had good or even high mean daily calcium intake (the current Finnish recommendation in adults is 800-900 mg/day). Calcium alone or in combination with vitamin D supplementation has been shown to decrease bone loss and fracture risk at the hip and spine in human clinical trials (Boonen et al. 2007, Tang et al. 2007). In children, total body and lumbar spine BMC increased with additional calcium in those children whose calcium intake at the baseline was below the recommended levels (Huncharek et al. 2008). The fact that no interaction of the C/T-13910 polymorphism and calcium index on peripheral bone traits was found in the present population may reflect our traditional food habits, including the relatively high amount of dairy products consumed in Finland. Nowadays, there is also a good availability of low-lactose and lactose free milk products in Finland. However, it seems that men with adult-type hypolactasia do not replace normal milk products with low-lactose and lactose free alternatives as often as women do (Laaksonen et al. 2009). This may lead to inadequate calcium intake and intestinal malabsorption of calcium. It was also reported earlier that Finnish subjects with lactase persistence tended to get more than the recommended amount of calcium from their diets (Laaksonen et al. 2009) and that they have 0.3 kg/m² higher BMI compared to the C/C carriers (Kettunen et al. 2010), both of which factors may have benefited their bone health. In addition, oestrogen production in the premenopausal phase could explain why no differences in radius and tibia were found in women. Oestrogen hormones increase cortical bone in long bone diaphysis and trabecular density at the axial skeleton in premenopausal women over men (Sievänen 2005).

Calcium intake was assessed with a comprehensive FFQ in 2007 which has been validated with a 3-day food record and was found to be a useful tool in epidemiologic studies (Paalanen et al. 2006). Adjustment for energy intake and the categorisation of the subjects into tertiles were used since reported amounts of food in FFQs are generally higher than in other dietary methods.

6.2.2 APOE -219 G/T and +113 G/C promoter polymorphisms

In Study II, women with the APOE promoter -219T/T and +113C/C alleles had the lowest CSI and BSI values at the distal sites of radius and tibia. They also had the lowest CSI at the tibial shaft. Men with the -219T/T and +113C/C alleles had, instead, the greatest total cross-sectional areas

and SSI at the radius. However, they also had the lowest cortical density at the radial shaft and CSI at the radial and tibial shafts. These results suggest that the APOE promoter -219T/T and +113C/C genotypes could be genetic risk factors for lower cortical bone at the radius and tibia.

Previously, the APOE -219G/T polymorphism has been linked to myocardial infarction, coronary heart disease, insulin resistance, ischemic stroke and atherogenic lipid and lipoprotein profiles (Lambert et al. 2000, Viitanen et al. 2001, Viiri et al. 2005, Viiri et al. 2006, Abboud et al. 2008). Subjects with hyperlipidaemia and atherosclerotic diseases may simultaneously have greater bone resorption due to an inhibition of osteoblasts (Parhami et al. 1997), increased osteoclast activity (Tintut et al. 2004) and upregulation of osteoclastogenic cytokine in T-lymphocytes (Graham et al. 2010). However, no hyperlipidaemia was detected among the APOE -219G/T and +113G/C genotypes in the present population. Serum glucose levels were lowest in women with the -219T/T and +113C/C alleles and highest among the G/G carriers, which may have confounded the results. Hyperglycaemia may e.g. induce changes in bone matrix and growth factors, which may induce bone fragility and increased fracture risk (de Paula et al. 2010). As fractures are more frequent in diabetic patients, the link goes also other way around. Glucose metabolism is known to be regulated by a bone-specific protein, osteocalcin, which increases insulin secretion and sensitivity via pancreatic β -cells. Additionally, there was a significant difference in alcohol intake, which was lowest in men with the -219T/T allele and highest in men with the -219G/T allele. The difference between these two genotypes was on average 1.8% of total energy intake. This might have influenced bone values in men. The diverse effects of ethanol on bone loss have been studied e.g. in patients using high amounts of alcohol over prolonged periods (González-Reimers et al. 2011). These alcoholic patients had significantly lower BMD values at the various sites of the body than controls and the lowest values were measured in cirrhotic patients. In a recently published meta-analysis, alcoholic liver disease was associated with approximately twofold greater risk of bone fractures; however, surprisingly, the risk of osteoporosis seemed to be somewhat lower than in controls (Bang et al. 2015).

Based on the gene-diet interaction analyses, a high intake of saturated fat measured as a sum of childhood and adulthood intakes seemed to be more harmful to bone health in women with the -219T/T and +113C/C alleles than in women with other allele types. In a recent study, non-obese mice set on a high-fat diet enriched with saturated fat had lower bone mineral content and density in total, and cortical and total density at the femur, than mice on a lower fat diet (Wang et al. 2016). In contrast, a high fat diet enriched with monounsaturated fat seemed to have a beneficial effect on femoral trabecular bone in these mice, suggesting the importance of fat type in diet.

In addition to the APOE -219 G/T and +113 G/C promoter polymorphisms, the association of APOE ϵ 2/ ϵ 3/ ϵ 4 genetic variation with radial and tibial bone traits was studied but only a few significant associations were found: women with the ϵ 4 allele had slightly lower cortical density and men, in contrast, had higher BMC at the tibial shaft. These results are in accordance with previous findings on this topic (Peter et al. 2011, Kim et al. 2016). The observed differences in cholesterol levels between the ϵ 4 carriers and the non-carriers could have, however, influenced the bone traits in

favour of subjects without the $\epsilon 4$ allele (Parhami et al. 1997, Tintut et al. 2004, Graham et al. 2010).

6.2.3 Significance of candidate gene results

According to the twin and family studies, variance in BMD is estimated to be up to 85% genetically determined (Ralston & Uitterlinden 2010). In addition, the heritability of bone loss was evaluated as being high, but in the case of fractures at an older age, the gene effect seems to decrease. Thus, studying the genetics of bones seems reasonable. In the present thesis, studying the genetic markers of various bone phenotypes at the radius and tibia produced additional new information since no previous publications existed that had studied the associations of lactase gene or APOE gene polymorphisms with pQCT-measured bone traits. In contrast to DEXA, pQCT used here can, for example, discriminate trabecular and cortical bones instead of areal bone mass, which yielded additional information on gene-bone associations. The SNPs used in this thesis were primarily selected because of their possible interactions with both dietary factors and bone traits. These SNPs also complement the GWAS approach since our understanding of the role of different candidate genes in bone biology is still limited. The identified SNPs in GWAS may not be the causal variants of bone phenotypes and, in addition to allelic heterogeneity, other genes in the same loci or nearby may also affect studied phenotypes (Estrada et al. 2012). It is also likely that the associations with small effect sizes and those arising from rarer gene variants may not manifest in GWAS. But since bone phenotypes, like osteoporosis, have a multi-factorial background, future genetic studies will surely focus on the contribution of multiple associations of different genes and gene-environment interactions towards various bone traits. From a nutritional perspective, this could mean, for example, that genetic variation in the requirements of nutrients could be taken into account in the future and more personalised dietary advice could be given in practice.

6.3 Physical activity and bones

6.3.1 Childhood and adolescence PAI

In Study III, frequent physical activity at the age of 9-18 years was associated with stronger tibial bone phenotype in adulthood at the age of 31-46 years. Those adolescent females who exercised the most had 47-67% lower risk of having below median BSI at the distal tibia and cortical area, BMC, CSI and SSI at tibial shaft compared to peers who exercised the least. In other words, girls who exercised frequently during their adolescent years were at a lower risk of having weaker bones many years later in adulthood. Similarly, males who were in the highest quartile of adolescent physical activity were at a lower risk of having below median bone area at the distal tibia, cortical area and CSI at the tibial shaft. Evidence from the previous pQCT studies is convincing and it seems that the greatest benefits of exercise for bone apposition are achieved during the early years of life (Kontulainen et al. 2002, Macdonald et al. 2007, Detter et al. 2014, Duckham et al. 2014, Nilsson et al. 2014).

The adolescent physical activity index used in this study was based on a short self-report questionnaire including items on participation in sport club training and competitions in addition to intensity and frequency of training sessions. The category 'frequent' complies with the recommendation of the American College of Sports Medicine for children and adolescents regarding bone health, i.e. impact activities and participation in sports that involve running and jumping, with sessions lasting 10-20 minutes and occurring at least 3 times a week (Kohrt et al. 2004). In the present population, favourite sports among females aged 9-18 years were cross-country skiing, running, walking, swimming, gymnastics, volleyball and cycling (Telama et al. 2005). In males, the most popular sports in 1980 were cross-country skiing, soccer, running, ice hockey, cycling, ball games and strength training. Many of these sports likely create moderate to high bone-loading forces and can therefore augment bone mineral mass and strength in children and adolescents.

Participants were also asked in a 2008 questionnaire whether they had sustained any fractures. Fractures classified as low-energy fractures were not consistently associated with childhood or adolescent physical activity for either gender. Among the characteristics of study subjects, it was found that females who were in the highest adolescent physical activity group had on average the highest intake of milk products and greater serum calcidiol levels at the baseline and the highest level of adult physical activity and a lower amount of alcohol consumption in 2007 compared to their less active peers. These differences have likely benefited their bone health (Weaver et al. 2016). Similar results were found in males according to mean serum calcidiol levels and adult physical activity scores.

When considering the limitations of the third study design, the design does not allow assessment of to what extent childhood and adolescent physical activities affect weight-bearing tibia since pQCT measurements have been done only once at the follow-up. It is also possible that physical activity in young adulthood may have modulated the results, which could be further studied separately. In addition, there might have been some factors during early development or genetic variants which were not controlled for in this study. As shown in the previous pQCT study, differences in bone mass, size, shape and estimated strength at the tibial diaphysis were established before puberty relative to their starting size (Wang et al. 2009). Although genes play an important role in determining the development of bones, lifestyle factors have also been shown to influence different bone outcomes in many studies, including this one. In addition, physical activity indices were based on data collected with a questionnaire, a method which is likely more prone to measurement errors and subjective interpretation than electronic activity monitors are (Janz KF 2006).

6.3.2 Pedometer-determined steps

In Study IV, women aged 31-46 years who exceeded 8765 daily steps had on average 1.1-1.7% larger total and cortical bone cross-sectional areas, 1.7-3.7% higher bone mineral mass and density, and 2.7-5.4% greater bone strength indices at the weight-bearing tibia compared to their less active peers. A greater number of daily steps was also associated with 0.5-3.8% higher

calcaneal ultrasound parameters in women. Similarly, in the non-weight-bearing radius, women in the highest tertile of daily steps had on average 1.7-2.3% larger bone areas, 1.7-2.2% higher bone mineral mass and 2.4-3.4% greater bone strength indices than their study peers. In men, however, the differences in calcaneal and radial bone traits were non-significant between the tertiles of daily steps. In tibia, the associations of daily steps with bone areas, BMC and SSI were significant but conflicting in men. These differences may reflect different physical activity habits between women and men in the present population. On average, the female study participants collected 764 steps more than men per day during the monitoring week. The higher bone values in women's radii within the highest tertile of daily steps may be due to Nordic walking, which was a popular leisure activity at that time in Finland. Since sticks are used in Nordic walking, it is a good exercise for the upper body too, including the arms. Men's results were mainly non-significant, and should be interpreted with caution. It may be, for instance, that men in the middle tertile of daily steps took their steps at higher intensity than the other men which then contributed to slightly higher bone area and mineral mass at the distal tibia. However, the pedometer which was used in the present study does not accurately measure the intensity of training and therefore this conclusion cannot be verified here.

The results presented may have some clinical relevance regarding the prevention of osteoporosis and fractures, as weekly walking has been associated with reduced hip fracture risk (Feskanich et al. 2002). However, in a meta-analysis of eight randomised controlled trials, no effect of walking interventions on lumbar spine bone mineral density was found whereas the effect on femoral neck was modest in postmenopausal women (Martyn-St James & Carroll 2008). The researchers stated that other exercises might be needed in addition to walking in order to decrease the risk of fractures in old age.

The previous positive associations between daily steps and bone outcomes have also been found in older people (Kitagawa et al. 2003, Kitagawa & Nakahara 2008, Foley et al. 2010, Shephard et al. 2017) and in children (Farr et al. 2011, Duckham et al. 2016). The mean number of daily walking steps varied between 8401-8842 among Japanese women aged 60-87 years (Kitagawa et al. 2003, Kitagawa & Nakahara 2008) whereas participants aged 50-80 years in Tasmania took an average of 8890 steps/day (Foley et al. 2010). In another Japanese study of women and men aged 65-84 years at the baseline, the mean number of daily steps was between 6896-7128 (Shephard et al. 2017). In Australian children aged 7-9 years, the mean daily steps varied from 7643 to 15495 (Duckham et al. 2016) and in American girls aged 8-13 years from 3603 to 16579 (Farr et al. 2011). When comparing these (bone) studies to the recommended number of daily steps, it can be concluded that older adults mainly exceed their recommendation of 6000-8500 steps/day but some children did not (recommended ≥ 12000 steps/day) (Tudor-Locke & Myers 2001). In the present study, women took on average 7827 steps/day and men 7063 steps, classifying them as 'low active' or 'somewhat active' (Tudor-Locke & Bassett 2004).

It was also found that participants in the highest tertile of daily steps had lower body weight and BMI than their peers with fewer daily steps. The proportion of women and men who smoked on a daily basis was also the lowest in the highest tertile of daily steps. In addition, participants with a greater number of daily steps had higher physical activity score and better maximal work output in

the cycle ergometer exercise test than their less active study peers. Additionally, mean serum calcidiol level was positively associated with daily steps. These health-related differences between the study groups may have confounded the results to some extent. Vitamin D, for instance, affects many physiological pathways of bone mineral homeostasis and it is also used as a supplement along with calcium in patients with osteoporosis (Bhattoa et al. 2016). In addition, it was shown earlier that in this population overweight children had 5-10% larger bone cross-sectional area at the radius and tibia later in adulthood (Uusi-Rasi et al. 2012). Furthermore, obese adults with a BMI over 30 had a decreased risk of low trabecular bone density at the distal sites of radius and tibia (Laaksonen et al. 2010).

The fourth study design has some limitations as it uses cross-sectional data to study the associations between step counts and peripheral bone parameters. Therefore, the outcomes between longitudinal steps and different bone traits are unknown. There is, however, prospective data on physical activity estimated with a self-report questionnaire which was earlier shown to predict the level of adult physical activity based on childhood physical activity (Telama et al. 2005). This physical activity questionnaire was also used in Study III, where frequent habitual physical activity at the age of 9-18 years was associated with higher tibial bone size and strength indices at the age of 31-46 years. Pedometer data could also be confounded by factors such as varying weather or participants' health conditions. As reported earlier, the most frequent reasons for non-participation and interruption of pedometer use were lost or broken pedometer (n=75), illness (n=30) and other reasons such as having an atypical day (n=22) (Hirvensalo et al. 2011). The pedometer itself has also some shortcomings as it only measures locomotion, mainly walking, accurately, which means that data on other types of exercise such as cycling and weight training should be gathered using alternative physical activity assessment methods.

7. SUMMARY AND CONCLUSIONS

(1) Lactase gene polymorphism was associated with trabecular density at the distal sites of radius and tibia in men. Men with the T/T-13910 genotype had on average 3% higher values than men with the T/C or C/C genotypes. No differences were found in other bone outcomes including low-energy fractures in either gender. In addition, the lactase gene-calcium interactions with the studied bone traits were non-significant.

(2) Differences in radial and tibial bone traits were contradictory and mainly non-significant between the carriers and non-carriers of the APOE ϵ 4 allele. In the groups of APOE promoter polymorphisms, women with the -219T/T and +113C/C alleles had the lowest bone strength values at the distal sites of radius and tibia. They also had the lowest cortical strength at the tibial shaft. Compared to the female G/G carriers, compressive and cortical strength were on average 2-8% lower in women with the T/T and C/C alleles. In men, cortical density at the radial shaft was ~1% and cortical strength at the radial and tibial shafts was ~2-3% lower among the -219T/T and +113C/C carriers than the G/G carriers. However, they also had the largest cross-sectional areas at the distal and shaft sites of the radius, which were ~4-5% greater than in men with the G/G allele. In addition, it was found that high longitudinal intake of saturated fat may result in smaller bone area at the radial diaphysis in women with the -219T/T and +113C/C alleles.

(3) The differences in adult tibial bone traits were non-significant in children aged 3-6. High physical activity scores at the age of 9-18 years were, instead, associated with stronger tibia in adulthood at the age of 31-46 years. Adolescent females with the highest physical activity levels had considerably lower risk of having below median bone strength, size and mineral content at the tibia compared to their less active peers. In adolescent males, similar differences were seen in tibial bone size and strength. The risk of low-energy fractures did not differ between the physical activity groups either in younger or older children.

(4) Women aged 31-46 years with daily steps over 8765 had on average 1-5.4% greater bone cross-sectional areas, bone mineral content and strength indices at the weight-bearing tibia and at the non-weight-bearing radius compared to their less active study peers. Additionally, the ultrasound bone parameters at the calcaneus were 0.5-3.8% higher among the more active women. In men, the associations of daily steps with the peripheral bone traits were mainly non-significant.

In conclusion, no major associations were found between the lactase gene polymorphism and peripheral bone traits in the present population. However, it cannot be excluded that subjects with lactase non-persistence might not be predisposed to lower bone density and fractures in other populations or age groups. The novel findings in Study II suggest that subjects with the APOE -219T/T and +113C/C alleles may be at a greater risk of lower cortical bone than the G/G carriers. In women, these homozygous APOE alleles were also associated with lower bone area at the radial shaft when the intake of saturated fat was high during the follow-up. The results of Study III support the idea that bone is sensitive to mechanical loading during pubertal growth and that

physical activity in childhood can influence bone strength in adulthood. In addition, habitual physical activity measured as daily steps seems to maintain skeletal health of peripheral bones in women over 30 years of age.

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Sanna Tolonen

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