UiT The Arctic University of Norway

Faculty of Health Sciences, Department of Health and Care Sciences

The influence of lifestyle on peak bone mass in Norwegian boys and girls between 15-19 years of age. The Tromsø study, Fit Futures.

Ole Andreas Nilsen

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Summary

Background: Osteoporotic fractures constitute a major health- and economic burden worldwide and because of an aging population the burden is estimated to rise. The individual consequences of fractures are severe. Norway has one of the highest fracture incidences in the world. The etiology of fracture risk at old age is less optimal bone mass accumulation in childhood and adolescence, rapid subsequent age-related bone loss or a combination of both. Therefore, peak bone mass (PBM) is a predictor of future fracture risk and to optimize bone accretion in young age identification of predictors of modifiable factors is essential.

Objectives: The aim of this thesis was to describe changes in bone traits during two years in late adolescence, investigate the degree of tracking of those bone traits and explore the associations between lifestyle factors such as body weight and snuff use and bone mineral density changes in Norwegian girls and boys between 15-19 years of age.

Methods: In 2010-2011 we invited all first comprehensive school students in Tromsø to the Fit Futures study and 1038 adolescents (93%) attended. We measured total body (TB), total hip (TH), and femoral neck (FN) areal bone mineral density (aBMD) as g/cm² by DXA (GE Lunar prodigy). Two years later, in 2012-2013, we invited all participants to a follow-up survey and 820 adolescents attended, providing 688 repeated measures of aBMD. We measured body weight and height and information on lifestyle were collected by questionnaires.

Results: Girls between 17 and 19 years of age were approaching PBM at femoral sites, while boys were still accumulating bone mass between 17 and 19 years of age. There was a high degree of tracking of bone traits during two years in late adolescence and drift between quartiles was limited. Body weight and body mass index (BMI) were associated with bone accretion in late adolescence, but in a healthy young population, the influence and clinical implications were limited. However, low BMI was associated with low aBMD and particularly among boys with low BMI, an increase in BMI could be beneficial for bone health. Use of snuff was associated with lower rate of bone accretion in boys, but its relation to maturation requires further investigation.

List of papers

This thesis is based on the following papers:

Paper I

Nilsen OA, Ahmed LA, Winther A, Christoffersen T, Furberg AS, Grimnes G, Dennison E, Emaus N. Changes and tracking of bone mineral density in late adolescence: the Tromsø Study, Fit Futures. *Archives of osteoporosis. 2017;12(1):37.*

Paper II

Nilsen OA, Ahmed LA, Winther A, Christoffersen T, Thrane G, Evensen E, Furberg AS, Grimnes G, Dennison E, Emaus N. Body weight and body mass index influence bone mineral density in late adolescence in a two-year follow-up study. The Tromsø Study: Fit Futures. *JBMR Plus. 2019 Aug 21;3(9):e10195.*

Paper III

Nilsen OA, Emaus N, Christoffersen T, Winther A, Evensen E, Thrane G, Furberg AS, Grimnes G, Ahmed LA. The influence of snuff and smoking on bone accretion in late adolescence. The Tromsø Study, Fit Futures. *Submitted.*

Abbreviations

aBMD: Areal bone mineral density

ANOVA: Analyses of variance

BA: Bone area

BMC: Bone mineral content

BMI: Body mass index

CI: Confidence interval

CV: Coefficient of variation

Δ: Delta, change

DXA: Dual-energy x-ray absorptiometry

FN: Femoral neck

PA: Physical activity

PBM: Peak bone mass

PDS: Pubertal development scale

RTM: Regression to the mean

SD: Standard deviation

SPSS: Statistical Package for the Social Sciences

TFF: The Tromsø Study, Fit Futures

TH: Total hip

TB: Total body

UiT: The Arctic University of Norway

WHO: World Health Organization

1 Introduction

1.1 Background

Osteoporosis, and its clinical expression, fragility fractures constitute a substantial and growing public health challenge worldwide [1]. If preventive measures are not taken, projections estimate the incidence to more than double during the next few decades [2]. Along with the high financial burden on society, there are often severe individual consequences such as pain, physical disability and loss of independence, reduced quality of life, increased morbidity and excess mortality [3, 4]. Fracture incidence vary significantly among populations [5, 6]. The Scandinavian countries are high fracture risk areas, and Norway has one of the highest incidence of hip- and wrist fractures worldwide [7, 8]. The reasons for these bone fragility disparities are not well-understood [9].

The primary cause of fragility fractures is compromised bone strength due to reduced amount of bone mass and diminished bone quality [10, 11]. Research and therapy have traditionally focused on mechanisms of bone loss and interventions following the first low-trauma fracture. However, attention to prevention has increased, and it is recognized that bone fragility late in life may have its antecedents in childhood and adolescence. In both girls and boys, bone mass increases substantially during growth and at the end of skeletal maturation the amount of bone mass peaks. Peak bone mass (PBM) is usually acquired between second and third decade of life and is followed by a consolidation phase before the gradual age-related degeneration begins [12]. Thus, bone mass levels in the elderly is a result of bone accrued during childhood and adolescence, less subsequent bone loss [13].

Adolescence refers to individuals between ages 10 and 19 and is a life phase in which the opportunities for establishing future health patterns are great [14, 15]. Approximately one third of PBM is determined by lifestyle choices and behavioural factors and at the end of adolescence, 95 % of PBM is achieved [12]. It has been calculated that 10% increase in PBM equals 50 % reduced risk of fracture later in life and an estimated delayed onset of osteoporosis by 13 years [16]. This makes optimization of PBM during growth a strategy for reduced risk of osteoporotic fracture and identification of predictors of PBM is essential.

The combination of an increasing elderly population and unhealthy lifestyle habits among children and adolescents may lead to increased incidence of osteoporosis [17]. Bone

accretion, and its determinants, from late adolescence into early adulthood, are understudied compared to time periods like childhood, puberty and post-menopause [12]. On this background, the focus of this thesis is bone mass and modifiable lifestyle factors in Norwegian girls and boys in their late adolescence.

1.2 Epidemiology of osteoporosis and fractures

1.2.1 Definition of osteoporosis and fragility fractures

"Osteoporosis" originates from Latin and literally means porous bone (os ="bone", porus ="an opening"). Over the years, there have been many definitions of osteoporosis because it is challenging to cover all its manifestations [18]. A frequently cited definition is from the 1993 Consensus Development Conference:

"…a systemic skeletal disease characterized by low bone mass and microarchitecture deterioration of bone tissue, with consequent fragility and susceptibility to fracture" [19].

The susceptibility of fracture is a complex matter, but one of its determinants is bone strength. Laboratory studies show correlation between the amount of bone mass and bones resistance to fracture [20, 21]. Bone mass predicts 60-70 % of the bone strength variation [16, 22]. Structural design and material composition explains the remaining variation. A number of properties like bone size, shape, physical properties of component material, micro damage accumulation, cortical thickness/porosity and distribution of trabecular and cortical bone all contribute to the strength of bone [11].

The National Institutes of Health Consensus Development Conference in year 2000 proposed an updated definition that included the concept of bone strength:

"A skeletal disorder characterized by compromised bone strength predisposing to an increased risk of fracture" [23].

Fracture is another hallmark of osteoporosis. This definition emphasizes **risk** of fracture as the clinical outcome. Osteoporosis is often referred to as "silent". Initial symptoms like low back pain, are not easily linked to skeletal disease and before the clinical manifestation of a fragility fracture, most people who are at risk unaware of it [2].

Osteoporosis has a syndromic nature and its etiology is multifactorial. Low bone mass is essentially related to failure to reach an adequate PBM, excessive bone loss, or both. The skeletal disorder broadly divides into two categories based on causation. **Primary** osteoporosis is when no underlying cause is identified, typically "natural" progressive bone deterioration caused by increasing age, lifestyle factors and/or menopause. S**econdary** osteoporosis is due to diseases and/or medical treatments [17].

Fragility fractures can be defined as low-trauma fractures due to forces generated by falls from standing height or lower. Kanis et al. defined osteoporotic fractures *"as occurring at a site associated with low BMD and which at the same time increased in incidence after the age of 50 years"* [24]. Apart from low bone mass, a leading mechanism of fragility fractures is excessive bone loading, i.e. falls. Ninety percent of all hip fractures are caused by a fall, and frequently in combination with low bone mass [25]. However, occasionally osteoporotic related fractures may also occur spontaneously [2].

Bone mass is an unspecified general term that often includes one of three following expressions (1). Bone mineral content (BMC; g), which is the most basic parameter, refers to the one-dimensional amount of bone mineral in grams, irrespective of width or depth (2). Bone mineral density (BMD), or areal bone mineral density (aBMD; g/cm^2) is a two dimensional measure of the quantity of minerals (BMC) per unit area of bone (BA) (3). Volumetric bone mineral density (vBMD; g/cm^3) is a three-dimensional measure comprising both width and depth. All three parameters can be elicited from bone densitometry techniques.

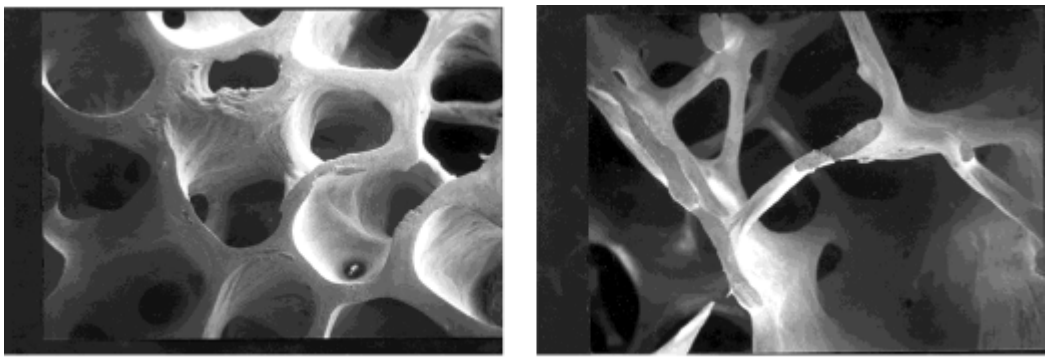
1.2.2 Dual Energy X-ray Absorptiometry, aBMD and diagnosis of osteoporosis

Bone's resistance to fracture is challenging to assess non-invasively. To date, aBMD measured by Dual Energy X-ray Absorptiometry (DXA) is recognized as the current "gold standard" for diagnosis of osteoporosis and fracture risk assessment [26]. The technique is a surrogate measure of bone strength and uses dual-energy x-ray beams to create a two dimensional image by the attenuation of photons by bone minerals as they pass through bone [27]. DXA is a widely used method because of its high precision, reproducibility, accessibility, low radiation, safety and low cost [28, 29].

There are numerous other approaches to assess properties of bone such as X-ray, metabolic assessment by bone turnover markers, volumetric BMD measured by Peripheral quantitative computed tomography and bone stiffness by Quantitative ultrasound etc. The two latter techniques can explore more subtle structural components of bone than DXA can, but are currently mostly used as research tools [30]. These aforementioned methods may be utilized in the diagnosis of osteoporosis, but the most common modality used is a DXA aBMD T-score.

The World Health Organization (WHO) classifies osteoporosis as a femoral neck (FN) aBMD value 2.5 standard deviations (SD) or more below the average score of young healthy Caucasian female reference population (20-29 years of age) [31]. This operational definition identifies individuals at greatest risk of fractures. The greater negative number, the lower aBMD and higher risk of fracture. In most situations, one SD equals 10-12% difference in aBMD [32]. Thus, an aBMD approximately 25% lower (i.e T-score of -2.5) than the average 30-year-old white female is the threshold for osteoporosis diagnosis. Although controversial, the diagnostic criteria for men is also based on the female reference population. This is based on evidence that the fracture risk at a given aBMD score is independent of gender [33]. "Established osteoporosis" is the preferred term for an individual with an osteoporosis diagnosis (T-score <-2.5) and one or more documented fragility fractures [34].

Figure 1



Normal vs. osteoporotic trabecular bone, from Dempster et al. [35], with permission of The American Society for Bone and Mineral Research.

Having a low aBMD score does not necessarily mean an osteoporosis diagnosis. The definition of osteopenia is a T-score between -2.5 and -1 SD. “Penia” means thinning and is not characterized as a disease. Normal aBMD is defined as a T-score above -1 SD [31]. aBMD T-score may be inappropriate for diagnosis in some cases. In children and adolescents, other diagnostic criteria and use of Z-score may be necessary, i.e. comparing with an average aBMD of individuals of same age and gender.

1.2.3 aBMD and fracture risk prediction

The rationale behind use of aBMD in diagnosis of osteoporosis is its ability to predict fracture risk. Low aBMD is a risk factor of almost all types of fractures in both sexes, but the predictive value depends on age and aBMD value. Low aBMD at a younger age is associated with a significantly higher gradient of risk [36, 37]. The relationship between aBMD and fracture risk is nonlinear. The risk increases exponentially as the aBMD decreases and small changes in aBMD can lead to greater than expected changes in fracture risk [38].

Measurements at the hip is the gold standard as it has the highest predictive value of the most serious outcome, hip fracture. In addition, measurements of the hip strongly correlates to most fracture types [39]. The predictive value of aBMD may be enhanced by taking other clinical factors like age, family history and use of medication into consideration. A prior fracture increases the risk of a subsequent fracture 2-5 fold [40].

Although at highest risk of fracture, the proportion of fractures attributable to osteoporosis (by the WHO criteria, ie. aBMD T-score < -2.5) is modest and ranges from <10 to 44 % [41]. The major burden of fractures in the overall population occurs at the osteopenic levels since more than half of all fractures in postmenopausal women occur in individuals with an aBMD score defined as osteopenia or normal. The main reason for this is that the majority of the population is within this T-score range [42-44].

1.2.4 Burden of osteoporosis and related fractures

Osteoporosis affects individuals in all age groups, both sexes and all races, but is more common in older people, women and Caucasians [45]. Advanced age is one of the major risk factors of osteoporosis and the risk of hip fracture increases substantially around 70 years of age [46]. Women (>50 years of age) have a four times higher rate of osteoporosis and a two

times higher rate of osteopenia compared with men [47]. The geographical disparities in osteoporotic related fracture incidences are substantial. Fracture rates in Northern America and Northern Europe are higher than in Asia and South America, and this diversity is only partly attributable to ethnicity [48-50].

Estimates suggest that there are 200 million people with osteoporosis globally [2]. In 2017, new fragility fractures in the largest five countries in Europe, (Germany, France, Italy, Spain and the UK) plus Sweden (EU6) were estimated at 2.7 million [51]. In the most recent estimate for the EU countries from 2010 (EU 27), approximately 22 million women and 5.5 million men between 50 and 84 years of age were affected by osteoporosis [52]. One in two women and one in five for men over 50 years of age will suffer a fracture during their lifetime [53]. In 2005, the lifetime risk for a fracture at age of 50 in UK, Sweden, Australia and US was estimated to 39-53 % and 13-22 % in women and men, respectively [54]. In Norway, 9000 people suffer from hip fracture each year (mean 80 years of age). That amounts to an average of one hip fracture per hour. Seven out of ten hip fractures is sustained by a woman [55]. In addition, 15000 forearm fractures and 23000 persons with vertebral fractures are registered annually [56].

The most frequent fragility fractures sites are proximal femur (18%), vertebrae (16%) and distal forearm (19%) [4]. Hip fractures are considered the most severe consequence of osteoporosis [40]. Breaking a hip at old age is life threatening, and men are more likely to die after a hip fracture. Excess mortality ranges from 8 to 36 % during the first year [57]. A study from Norway shows that mortality within the first year post hip fracture was 21% for women and 33% for men, and the excess mortality remained significantly increased for 12 years [58]. Only half of hip fracture survivors regain their pre-fracture status judged by ability to walk and need for aid [54].

The economic consequences of fractures are huge. Total cost of osteoporotic fractures within the EU6 were stipulated to €37.5 billion in 2017 [51] . In Norway, the annual cost of hip fractures is estimated to be 7-9 billion NOK and the price of a single hip fracture is estimated to be 550 000 NOK the first year [55].

Fracture incidence rates seems to be declining in many western countries [59] and recent years there has been a 1.5 % annual decline in hip fracture incidence in Norway [9, 60, 61].

The reason for this decline is still unclear. However, the overall burden is likely to increase [62]. According to the International Osteoporosis Foundation, trend analysis project that demographic changes with longer survival and a higher proportion of older people worldwide will at least double the incidence of bone and joint related diseases in the next 20 years. Unless appropriate preventive measures are taken the incidence of hip fracture is estimated to increase by 240% in women and 310% in men by 2050 [2]. Other estimates indicate that 1.66 million hip fractures in 1990 will rise to 6.26 million in 2050 [63].

1.3 Bone tissue

1.3.1 Function and components

Bone is a complex dynamic connective tissue undergoing constant renewal throughout life [64]. The human body comprises 305 bones at birth, but because some bones combine during growth, the adult skeleton consists of 206 bones. The skeleton serves **mechanical** and **metabolic** functions. Bones have mechanical properties for protection of vital inner organs and the brain, support against gravity and locomotion as they act as levers for muscles to pull on as a framework for movement [27]. Bone tissue is very metabolically active, highly vascularized and acts as the primary site for hematopoietic cell maturation [65]. Both red- and white blood cells originate and develop in the bone marrow. Bones are crucial in mineral homeostasis and serve as a depot for important minerals like calcium, phosphate, magnesium, potassium and sodium. Bones contain approximately 99 % of the body's calcium stores. The serum mineral levels are kept at a narrow range and various minerals are released into the bloodstream when needed and stored if concentration is too high [66].

There are especially three hormones critical in the regulation of calcium and phosphate: parathyroid hormone, which increases serum calcium; calcitonin, which has the opposite effect of parathyroid hormone and inhibits bone breakdown; and calcitriol (vitamin D) that promotes absorption of dietary calcium from the gastrointestinal tract [32]. Yellow bone marrow are responsible of storage of fat and adipocytes. Lastly, bone also maintain other functions like short time electrolyte balance and acid/base balance [64].

Imbalance between competing responsibilities of bone can potentially compromise important functions, i.e. situations with repeatedly high demands of calcium in the bloodstream may be detrimental to bone strength [67].

1.3.2 Structure

The skeleton weighs approximately 10 kg and accounts for about 15 % of the body weight [2]. The axial skeleton includes the head and the trunk, while the limbs and pelvic girdle make up the appendicular skeleton. Long bones make up most of the limbs and its typical structure is the diaphysis (the shaft), the metaphysis, (growth plate) and epiphysis (the ends). The periosteum covers the outer surface except articular surfaces and contains osteoprogenitor cells, blood vessels and nerves. A thin membrane called the endosteum covers the medullary cavity, the hollow space within the diaphysis [65].

Essentially, the building blocks of bone are collagen fibers, reinforced by minerals. The inorganic mineral matter make up 60 % of total weight of bone, 8-10 % is water, while organic matrix constitute the remaining. The inorganic matter consists predominantly of calcium phosphate crystals, 85 % hydroxyapatite (Ca_{10} $(PO_4)_6$ (OH_2). The organic matter is mainly type1 collagen and non-collagenous proteins (98%), and the remainder is cells. These materials are fashioned into two types of osseous tissue: cortical- and trabecular bone [68].

Figure 2

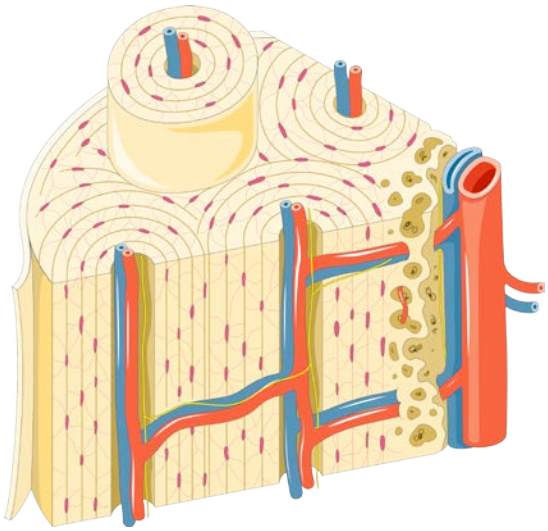


Illustration of bone structure. Periosteum covering the outer surface, longitudinally oriented osteons, Haversian canals containing blood vessels, and trabecular bone located interiorly. Creative commons license.

The majority (80%) of the skeleton consists of compact and dense **cortical bone** found in the hard outer layer predominantly in diaphyseal regions of long bones. The functional unit of cortical bone is the osteon [64]. Osteons are longitudinally oriented and consists of successive concentric layers called lamella. These layers surrounds the central canal (Haversian canal) that contains small blood vessels, nerves and lymphatic vessels. Volkmann's canals run perpendicular to Haversian canals and connects osteons with outer blood vessels [65].

Trabecular bone is cancellous, honeycomb-like spongy bone present in the interior of the axial skeleton and in epiphysis of long bones. Because of the characteristic network of lamellar plates and rods, this osseous tissue has lesser density and directional homogeneity than cortical bone does. There are no vessels within trabeculae and the bone is supplied by diffusion from bone marrow [25].

The two types of bones has different properties because of its composition, structure and spatial distribution of minerals. Because function and demands of physical attributes vary from bone to bone and within bone, the proportions of the two different types vary extensively throughout the skeleton and the multiscale hierarchical macro- and microstructure optimizes their properties and function [65].

Bones have contradictory needs. They must be strong to not break, but also be lightweight in order to move easily. They have to be rigid and stiff to resist deformation, but also flexible to absorb energy from tension and compression without structural failure [27, 69]. The combination of inorganic and organic matter makes bones both strong and resilient. Collagen give bones tensile strength by its cross-linking profile and mineralization strengthens the mechanical resistance [70]. It has been calculated that minerals provides 80-90 % of the compressions strength in bone [71].

In long bones and at skeletal sites that needs to resists bending and rotational forces, the stiffness and cortical bone is favored. The femoral neck is approximately three quarters cortical bone [32], while the vertebrae is more of a shock absorber and consists mainly (60-70%) of spongy trabecular bone [11, 68]. For lightness, the principle is to minimize the amount of mass needed for appropriate bone strength. The porosity of trabecular bone is 40-95%, compared to cortical 5-20 % [69]. The bone size and the position of the cortex related to the neutral axis determines strength, as the bending strength of a bone increases

proportionally to the fourth power of the radius [11]. This means that doubling the diameter of a hollow bone without increasing mass increases its strength eightfold [72].

1.3.3 Cellular composition

There are three primary types of cells in bone.

Osteoblast are specialized bone-forming cells responsible for production of the organic matrix. They secrete and synthesize collagen, and contribute to the mineralization process by initiating the calcification [73]. As osteoblasts mature, they have three different pathways: remain osteoblasts, become osteocytes or become “resting osteoblasts” and form bone lining cells on the surface of bone [74].

Osteocytes are mature osteoblast and make up more that 90 % of the bone cells in the adult skeleton. Imbedded in mineralized bone they occupy the “empty spaces” in bone matrix lacunae, communicate with each other through their long dendritic processes and form the intricate lacunar canaliculi network. [73]. In addition to nutrition and oxygen supply, osteocytes play a key role in mechanotransduction. This process transfers mechanical loading into electrochemical activity. Information about magnitude and distribution of stress and interstitial fluid flow are passed on to bone cells that subsequently maintain and modify bone mineralization [75].

Osteoclast are absorption cells. They are derived from multiple stem cells and have many nuclei. Osteoclasts operates on the bone surface in hollow depressions called Howship’s lacunae, and digests bone with an enzyme called tartrate resistant acid phosphatase using their ruffeled boarder facing the matrix. Acids separate minerals from proteins and disrupt the bonding forces of bone [74].

1.3.4 Bone remodeling

Remodeling is a cellular mechanism that maintain and repair bone. The process occur throughout life and is orchestrated by coordinated activities of osteoblasts and osteoclasts. This cellular link, known as coupling, is a continuous, tightly regulated process of bone tissue breaking down (resorption) and regenerating (formation) to prevent accumulation of micro damage. Micro-cracks due to loading are removed and replaced with new bone, ensuring the

integrity and strength of bone. It is a balanced sum of two processes and replacement normally occur in equal proportions and leads to minimal change in architecture [67].

Most remodeling sites are random and takes place in the basic multicellular unit. Ten to 20 % of the skeleton is replaced each year while the total volume is maintained. This means that we have a new skeleton each 7-10 years [64]. The remodeling cycle takes 3-6 months and have 5 stages: activation, resorption (2-4 weeks), reversal, formation (4-6 months) and termination [73, 76].

The surface of trabecular bone is 10 times larger than cortical bone and the turnover rate is five to ten times higher. This indicates that trabecular bone responds easier to mineral metabolic demands, but it also makes it vulnerable during life phases of bone mass reduction. The combination of weight bearing and spongy bone skeletal cites are at risk: the lumbar vertebrae, hip and the distal forearm (falling) [64].

Because women have smaller and thinner bones, earlier onset and accelerated bone loss around menopause, they tend to have earlier onset of osteoporosis compared to men. The rapid decline in estrogen production increases the lifespan of osteoclasts (estrogen promotes apoptosis) and this leads to net loss of bone mass due to an imbalanced remodeling process with increased resorption and inadequate deposition [73].

1.3.5 Bone growth and modeling

During childhood, and especially puberty, the skeleton changes substantially and the predominant process behind this is **modeling** [73]. Skeletal development follows a specific pattern according to age and is a coordinated action between resorption and deposition according to a genetic program [77]. In the modeling process bone resorption and bone formation are uncoupled. It involves destruction and putting bone in new places, moving bone surfaces in tissue space, changing its size, density, shape and architecture [67]. Modeling starts with fetal growth and ends with epiphyseal fusion in the twenties [12]. Longitudinal growth is driven by bone formation at the diaphysis side of the epiphyseal plate, while appositional growth occur because of periosteal deposition and endosteal resorption. Periosteal apposition increases the diameter of the bone, while endosteal resorption excavates the medullary cavity and shifting the cortex away from the neutral axis [73].

There are two different processes behind formation of bone tissue: **intramembranous ossification** is the process of forming bone from fibrous membranes and mainly occur in flat bones in the skull, mandible and clavicle. Rudimentary formation and longitudinal growth of long bones is primarily caused by **endochondral ossification**, which creates bone tissue from cartilage.

The quantity of bone minerals is approximately 70-90 g at birth and eventually mounts up to 2400 g and 3300 g in women and men, respectively [78]. The skeleton grows slowly and consistently in childhood, then the accumulation of bone mass increases rapidly in puberty throughout the growth spurt. Both height velocity and bone mineral accretion rate peaks during puberty [79]. Roughly, 40 % of adult bone mass is accrued during the four years surrounding the growth spurt. This is as much bone as most people lose throughout four decades later in life [10, 80]. Pubertal bone growth is due to increased bone size rather than increased bone mineral density and peak height velocity precedes peak bone mineral accretion by 6 to 12 months. This lag and imbalance between size and mineral accumulation makes the skeleton susceptible to fracture for a short period of time [81].

aBMD continues to rise to final stature and beyond, and men continue to accrue aBMD for several years longer than women do. By 4 years following peak mineral accretion, 95 % of adult bone mass is accumulated [10, 82]. Between the second and third decade of life accretion flattens depending on the skeletal site and the consolidation phase begins [12, 83]. Skeletal characteristics influencing structural strength like cortical density and size continues to increase into the third decade. Certain bones, including femur, continue to expand even after cessation of linear growth [84-86].

The purposes of modelling and remodeling during growth and adulthood are not the same. In childhood and adolescence, the purpose is construction and the attainment of peak bone strength, i.e. bone formation is higher that bone resorption. Once the skeleton has reached maturity and longitudinal growth has ended, regeneration continues to maintain strength. In the consolidation phase the processes balances out, while during age-related bone loss, bone resorption is higher that formation leading to net bone loss [27].

1.3.6 Pubertal maturation and sex differences in bone development

Until puberty, the bone maturation and skeletal growth are more or less equal between girls and boys. At the onset of puberty, endocrine and hormonal differences leads to disparities in development [87]. Boys tend to have equal or higher BMC and aBMD than girls at the end of puberty depending on skeletal site, but at a later age and mostly due to greater bone size [47]. Cortical thickness in girls and boys are similar. The most apparent difference is the position of the cortex related to the long axis of the bone. In girls, increased estrogen production throughout puberty suppresses periosteal apposition and endosteal resorption leading to a smaller skeleton. The bones diameters are smaller, but not necessarily less dense [11]. Compared to boys, girls have a larger trabecular area and this may enable easy access to minerals during pregnancy without compromising bone strength [81].

Studies suggest that, in girls, the highest BMC accumulation rate takes place from 12-15 years of age, compared to 14-16 years of age among boys. Bone accrual levels off in girls and boys by the age of 16-18 and 17-20 years, respectively [88].

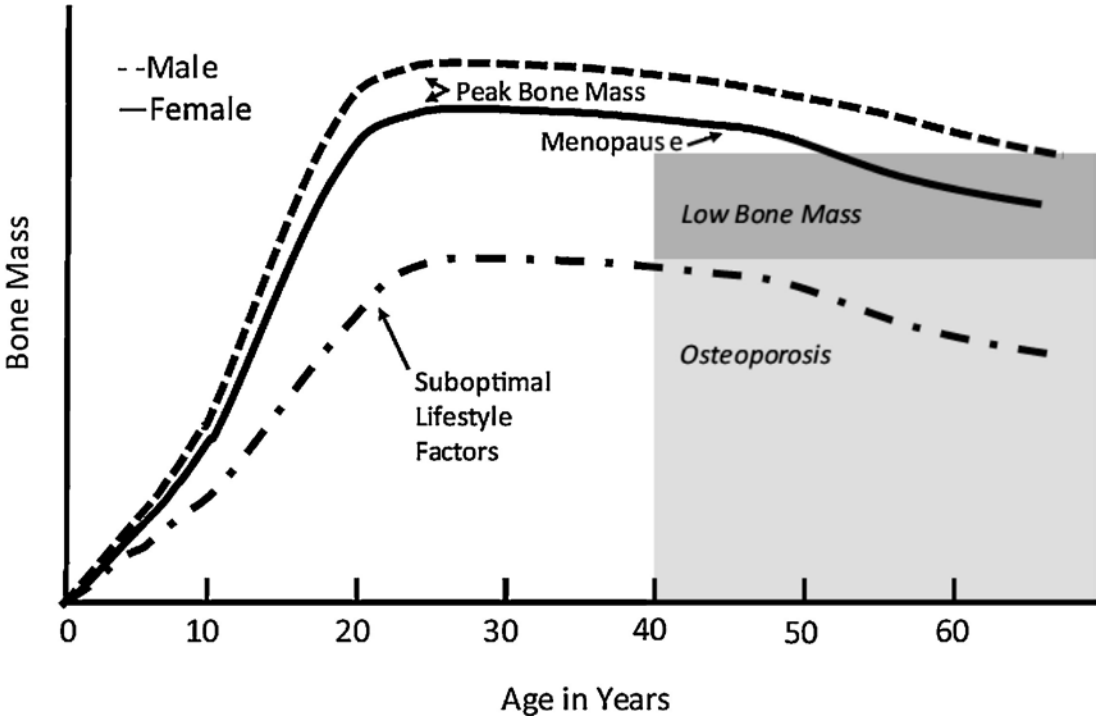
1.4 Peak bone mass and its determinants

PBM can be defined as the highest amount of bone mass achieved at skeletal maturation [10]. Age-determination and timing of PBM has been under some controversy, but the prevailing view is that PBM occurs by the end of the second or early in the third decade of life, depending on gender and skeletal site [10]. The concept of PBM has different nuances. Individually it refers to the optimization of the genetic potential for bone mass, while at a population level, PBM is achieved when age related changes level off and a stable skeletal state has been attained. Even more broadly, it could capture peak bone strength as well. PBM is a widely recognized determinant of osteoporosis [12]. During growth, the normal range in values of bone traits around the age-specific means are large (10-15 %) compared to those related to rate of bone loss (1 %). Thus, it has been advocated that the determinants of accretion is likely to be at least as, and maybe even more, important as those preserving bone mass throughout life [77, 89].

Heredity and genetics explain 60-80 % of the variance in PBM, while hormonal and environmental factors make up the remainder [90]. The genetic influences declines with age and the contribution of environmental factors increase [91]. **Non-modifiable** determinants of

PBM include gender, age, race, height, hormonal status, disease and genetics. **Potentially modifiable** factors mainly relates to lifestyle and include physical activity, nutrition (calcium, vitamin D), contraceptive use, alcohol consumption and recreational drugs like smoking and use of snuff. Body weight, body composition and BMI may be considered as a hybrid of the aforementioned categories as they are modifiable, but there is a considerable hereditary component as well. Potentially modifiable factors are most influential during growth and an unhealthy lifestyle can put individuals at risk of less than optimal PBM [12, 77].

Figure 3



Bone mass across the lifespan with optimal and suboptimal lifestyle choices. Reprinted from Weaver et al [12].

The lifelong significance of PBM in relation to fracture risk is not fully understood. The clinical importance of maximization of PBM depends on to what degree bone mineral status in younger years tracks into old age [12]. Tracking refers to the stability of bone traits within a distribution over time and is a critical assumption behind the importance of PBM. Some studies suggest a high degree tracking throughout childhood and adolescence [92-96], while other creates doubt about the value of early identification of individuals at risk of low bone

mass [97-99]. The degree of tracking seems to depend on skeletal site, trait and duration of follow-up time. Imperfect tracking indicate the possibility of bone mineral status alternation and thus, enhances the need for lifestyle determinant detection. Tracking of bone traits post-PBM is also documented [100-102]. Better knowledge of the lifelong importance of PBM requires expensive and time-consuming longitudinal studies with follow-up from childhood into old age. Though, the feasibility of such studies has been questioned [103].

1.4.1 Body weight and body composition

Bone mass is closely related to body weight and –height because the skeleton needs to be appropriate for body size [67]. Body weight is a major determinant of bone mass explaining a large proportion of the variance at a population level in the adult population [85]. As height and weight are interrelated, body mass index (BMI) is a common tool of weight adequacy considering a person's height. There is evidence of a nonlinear relationship between fracture risk and BMI in older individuals, with a marked increase in risk from normal to low BMI (<25) [104]. The prevalence of osteoporosis (low BMD) is negatively associated with BMI. In women, there is a decrease from 45 % in BMI<18.5 to <1% in obese (BMI>30) [105].

The prevalence of obesity among adolescents (BMI>30 kg/m^2) is rising in Norway [106] and worldwide [107]. Studies suggest an increased fracture incidence in obese children [108]. Several studies have investigated the relation between weight and bone in childhood and adolescence and reports on whether excess weight interfere with bone mineral accumulation are controversial [108-113]. A resent systematic review and meta-analysis including 27 studies and 5958 individuals concluded that overweight children had significantly higher aBMD compared to children of normal weight [114]. Other studies have found lower than expected spinal BMC and bone area in obese children and adolescents [115].

Individuals of tall stature naturally have higher BMC than shorter do. Baxter-Jones et al. reported that height accounted for nearly 70 % of prediction of total body BMC at peak height velocity in a study of bone mineral accretion among eighty-five boys and 67 girls measured annually for seven consecutive years in a mixed longitudinal design from the age of 8 to 19 years. Body weight (lean mass and fat mass combined) had a contribution of 29 percent [83].

Body weight could exert an effect on bone accretion through diverse direct, or indirect, underlying mechanisms related to both mechanical forces and hormonal status. Growing bone is highly responsive to mechanical loading (as well as unloading). Early in the 19th century, Wolff proposed a hypothesis that trabecular bone adapted and aligned with the stress directions though a self-regulated structure-function relationship process [69, 116]. Frost further developed the theory with the hypothesis that if peak strain is higher or lower than normal, compensatory mechanisms are initiated. The mechanostat theory postulates that these mechanisms removes bone where mechanical forces are low and adds bone at skeletal sites where demands are high [72]. Load determines the structure of bone and its form follows function [69]. This way, the system avoids both unnecessary bone and catastrophic failure. However, this also makes bone vulnerable for atypical loads, like falls inflicting of-axis loads on the trabecular bone, i.e. falling sideways and breaking the femoral neck [67].

BMI and body weight are commonly used metrics in relation to bone research, but have inadequacies when exploring the exclusive influence of adiposity and muscles. Individuals with equal BMI may have very different body composition and measures that are more refined are available. In addition to gravitational load on weight-bearing bones, increased muscle mass is likely to contribute to the positive influence of high BMI on bone. The impact of lean mass on bone during growth [117, 118] and in adults [119] is established, while the significance of fat mass is more controversial and appears to vary with age from adolescence to adulthood [109]. The mechanostat mechanism of fat mass seems to be limited to weight and gravity. Obesity may be related to bone thru increased mechanical loading (both gravity and higher lean mass), diet and due to excess fat mass. However, fat mass could also exert an endocrine function [67]. Hormonal changes attributed to adipose tissue may be the reason why obesity is associated with compromised cortical bone quality in young individuals and may not always protect against osteoporosis in old age [120]. The influence of body weight on skeletal health may also be modified by sedentary behavior and other lifestyle factors.

1.4.2 Pubertal development

There are considerable maturational differences between adolescents at the same chronological age [88] and timing of bone mineral accretion is closely related to pubertal development [81]. The onset of puberty at older age is associated lower PBM, particularly in girls [121]. aBMD and BMC scores at 20 years of age appear to be lower in individuals with

late onset independent of bone pre-scores and duration of puberty. Furthermore, there are indications that maturational timing are most influential at the TB [79]. Nevertheless, the long-term importance of pubertal timing on PBM is not clear [12, 122]

1.4.3 Physical activity and mechanical loading

There is strong evidence of the impact of physical activity on PBM, even at a recreational level [12, 123]. Physical activity is closely related to lean mass and its impact on bone is partly explained through mechanostat theory (loading by gravity) and association with muscles (by the attached tendons) [124]. The largest strains on the skeleton come from muscles forces, not gravity [125]. Studies comparing the playing arm and the non-playing arm in tennis players support mechanostat theory and suggest that size and structure adapts to loading during growth [46, 126-128]. E.g. the humerus exhibits approximately 40% more cortical bone on the arm that holds the racquet [129]. Some forms of physical activities has been shown to be more "osteogenic" than others. During walking, weight-bearing bones in the lower limbs are subjected to a mechanical load of approximately 1.5 times the body weight with a one-second interval [67]. To maximize the benefits for bone the activity should be weight-bearing, dynamic, of moderate to high in load magnitude, include odd- or non-repetitive in load direction and be applied quickly [12]. Furthermore, it is important that the mechanical loading of the activities exceed an individual given threshold set by habitual activity, maturation and other factors.

1.4.4 Tobacco use

Smoking is widely regarded as detrimental to bone in the adult population at all skeletal sites, with an observed clear biological gradient related to dose and duration of exposure. Smoking heightens an individual's fracture risk through both reduced bone mass and bone mass independent factors. Potential pathophysiological mechanisms of the adverse effects of tobacco on bone are poorly understood [130-132]. The evidence on its deleterious effect on PBM is not as compelling. Studies report both statistically significant deficits in aBMD [133-139] and no differences according to smoking [140-143]. Most studies of the associations between smoking and bone have methodological issues, but large studies of military recruits provide some evidence of deleterious effects. Generally, the effect sizes in the studies are small; however, the accumulated effect over time could be significant [12]. Winther et.al.

found a cross-sectional association between reduced aBMD and smoking in Norwegian boys 15-17 years of age [136].

In Norway, an additional public health-related challenge in terms of tobacco use and PBM has emerged. Use of snuff (Swedish snus: smokeless, oral tobacco) has been increasing among adolescents in Norway for several years, while traditional smoking is decreasing [144]. WHO regards smokeless tobacco as a significant part of the overall use of tobacco [145]. There is a great diversity of products, with a range of health hazards. The cross-sectional relationships between of use of snuff and aBMD among Norwegian adolescents have previously been explored and no significant associations were reported [136]. Apart from this study, the influence of use of snuff on growing bones is hardly described.

1.4.5 Other determinants

There is a wide range of determinants of PBM identified. The evidence of the influence of alcohol consumption on bone in adolescence is conflicting. Both positive [146], no associations [134] and negative associations [140, 147] between aBMD and alcohol have been reported. Use of combined hormonal contraceptives (CHC) and progestin-only methods has been shown to be associated with skeletal deficit in girls, but the findings remain controversial [148-152]. Nutrients widely regarded as beneficial for bone health are calcium (dairy consumption) and vitamin D. Access to calcium during growth influence both bone accumulation and fracture risk. Intestinal absorption of calcium depends on adequate vitamin D levels [153]. The influence of other micronutrients, dietary components and macronutrients like fat and protein, are not compelling [12].

1.5 Assessment of bone mineral density in children and adolescents

The interpretation and reporting of results from DXA –scans in children and adolescents differ from those in adults. Because of the large variations in bone size, bone densitometry are often difficult to interpret [154]. In growing individuals, current clinical recommendations from ISCD is to compare scores with reference values for the same sex, age and race (Z-score). Because PBM has not occurred yet, it is inappropriate to use a T-score. DXA scans are two dimensional and unable to detect bone depth. Therefore, aBMD estimates are size dependent and individuals of short stature and smaller bones would get falsely low scores. The spine and total body less head are the preferred scanning site according to the ISCD.

There are concerns regarding the precision of DXA-results at the hip because of the variations in development of skeletal landmarks. However, hip scans can be performed from 11 years of age when software better can detect the region of interest, and in later adolescence this issue may not be as significant [103, 155].

Figure 4

Illustration of DXA-scanning.

2 Aims of the thesis

Individuals with high PBM after adolescence might have a protective advantage related to skeletal health and future fracture risk. A better understanding of the factors that maximize acquisition of bone mass during growth is an important public health strategy to improve osteoporosis related outcomes. There is a paucity of data on adolescent health behaviour and bone accretion from late adolescence through early adulthood. This thesis explores factors connected to PBM and describes the influence of potentially modifiable lifestyle factors on this achievement. With the prior chapter as a background the aims of the present thesis are to:

1. Describe changes in- and explore the degree of tracking of aBMD levels over two years in adolescence, i.e. find out if participants mainly remain in their original aBMD quartile between the age of 15-17 and 17-19 years.

2. Explore the associations of baseline body weight/BMI and body weight/BMI changes over two years on changes in aBMD (ΔaBMD). A question of clinical interest is to what extent body weight gain increase peak bone mass acquisition in those with low BMI at baseline.

3. Evaluate if lifestyle factors such as use of snuff and smoking influence ΔaBMD peak bone mass acquisition in adolescence.

The main outcome of this thesis is aBMD, and particularly ΔaBMD. However, parameters of BMC and BA are frequently reported to support the understanding of growth and bone accrual, especially when it complements or deviates from the findings of aBMD.

3 Material and methods

3.1 Study design and samples

The Tromsø study is an ongoing population-based study initiated in 1974. The study consists of seven health surveys conducted in the municipality of Tromsø [156]. This thesis utilizes data from The Tromsø Study: Fit Futures (TFF), which is an extension and the youth cohort of the Tromsø study. TFF is a collaboration between the University Hospital of North Norway, UiT The Arctic University of Norway and the Norwegian Institute of Public Health and intends to compliment The Tromsø study with research on adolescents' lifestyle- and health.

The first wave of TFF was initiated in 2010-2011. All first-year students from all eight upper-secondary schools in both academic, sports and vocational educational programs from the two neighbouring municipalities Tromsø and Balsfjord were invited to Fit Futures I (TFF1). The overall attendance rate for upper-secondary school in this region of Norway is more than 90% [157]. Out of the 1301 potential students that were registered to start, 184 individuals were school dropouts, hindered by disease or individuals that we were not able to contact. The invited cohort mainly born in 1993-1994 included 1117 participants. 508 girls and 530 boys attended the survey providing an attendance rate of 92.9 %. Ninety five percent of the participants were between 15-18 years of age.

In the second wave two years later, in 2012-2013, all third year upper-secondary school students in the same schools were invited to a follow-up survey, Fit Futures 2 (TFF2). Participants of TFF1 not attending third year at comprehensive school two years later due to relocation etc., were also re-invited in TFF2. A total of 820 adolescents attended, providing 688 repeated DXA measures of bone traits (66% of the original cohort).

The study population varies in the three papers due to missing variables and inclusion criteria.

3.2 Ethics

The study protocol for TFF1 was approved by The Norwegian Data Inspectorate 27.07.2010 (Ref. 07/00886-7/CGN) and the Regional Committee of Medical Research Ethics (REK-Nord) 16.09.2010 (Ref. 2009/1282-23). The study protocol for TFF2 was approved as an extension of the prior approval by the Data Inspectorate 31.10.2012 (Ref. 07/00886-15/EOL).

Paper I and II were approved by REK-Nord 27.08.13 (Ref. 2013/1459/REK nord) and paper III 19.09.2019 (Ref. 2019/31193/REK nord).

All participants received a descriptive information leaflet regarding the survey in advance and gave written informed consent according to the Declaration of Helsinki [158]. Participants below 16 years of age had to bring written consent from their superiors to attend the survey. After completion of the surveys, participants were given a compensation in form of a 200 NOK gift voucher.

3.3 Measurements

3.3.1 Measurements of aBMD and BMC

We measured total body (TB), total hip (TH), and femoral neck (FN) BMC (g) and aBMD as g/cm² by DXA (GE Lunar prodigy, Lunar Corporation, Madison, Wisconsin, USA) and analysed them by Encore paediatric software v. 13.4 [159]. We used auto-analysis mode and default region of interest. We used the same densitometer in both TFF1 and TFF2. Trained technicians in the University Hospital's research lab performed the measurements according to manufacturer's procedures, and the DXA scanner was calibrated daily according to the same protocol in both surveys and between surveys. Participants were asked to remove all jewelry, bracelets, metallic objects, eyeglasses and such, and scanned in supine position in light clothing. A wedge were used to ensure correct hip position. DXA scans were subsequently assessed for abnormalities and diverse artifacts that might influence BMD results. Primarily, we used measurements of left hip at both femoral sites. In 15 cases, the left hip measurement was missing or erroneous, and then the right hip was used in replacement. The same hip was used in both baseline and follow-up for comparison.

In paper I, we converted BMC and aBMD measures into sex- and age-standardized internal z-scores based on the distribution of the study sample.

3.3.2 Anthropometric measures

We measured body height and body weight to the nearest 0.1 cm and 0.1 kg on a Jenix DS 102 Stadiometer (Dong Sahn Jenix, Korea), following standardized procedures according to The Tromsø Study. Participants were wearing light clothing, no shoes or metallic objects.

BMI was calculated as weight divided by height squared (kg/m^2). In paper II participants were stratified into weight categories using Cole's BMI cut-off [160]

3.4 Self-reported questionnaire

Through the data program "Questback", a web-based general health and lifestyle questionnaire was used to collect information on lifestyle, nutrition, wellbeing and health problems.

3.4.1 Use of tobacco

The questions on "Do you smoke?" and "Do you use snuff?" had three alternatives: "No, never", "sometimes" or "daily". If the response were "sometimes" or "daily", participants were asked follow-up questions on frequency and duration.

The questions were: "If you use snuff sometimes, how many snuff portions do you usually take per week?" Alternatives were "One or less", "2-3", "4-6", "7-10" and "More than 10". For daily users the subsequent question was: "If you use snuff daily, how many snuff portions do you usually take per day?". Alternatives were "1", "2-3", "4-6", "7-10" and "More than 10". The age of onset of use of snuff, were elicited by the question: "How old were you when you started to use snuff?" The 8 alternatives were: "Below 12 years", "12 years", 13 years",14 years", "15 years", "16 years", "17 years", "18 years" and "19 years or above". In the TFF2 questionnaire, one alternative to the questions on snuff and smoke was added: "In the past, but not now".

3.4.2 Physical activity

Physical activity was assessed by the questions from the modernized Saltin-Grimby Physical Activity Level Scale (SGPALS) or Gothenburg instrument [161]. The participants were asked to grade their time spent on physical activity in leisure time in an average week during the last year.

If their activity varied much, for example between summer and winter, then they were asked to give an average. The question referred only to the last twelve months. The alternatives were:

1) Reading, watching TV, or other sedentary activity? 2) Walking, cycling, or other forms of exercise at least 4 hours a week? 3) Participation in recreational sports, heavy outdoor activities, snow clearing etc.? 4) Participation in hard training or sports competitions, regularly several times a week?

3.4.3 Pubertal status

Pubertal status for girls was determined through the following questions: "If you have started menstruating, how old were you when you had your first menstruation?". Participants were given the opportunity to respond in years of age, and more specifically month. Answers were categorised into "Early" (<12,5 years at menarche), "Intermediate" (12,5 – 13,9 years) or "Late" (> 14 years) sexual maturation.

Pubertal maturation in boys was examined according to Pubertal Developmental Scale (PDS). The boys rated secondary sexual characteristics as growth spurt, pubic hair growth, and changes in voice and facial hair growth on a scale from 1 (have not begun) to 4 (completed). We summarized the score and divided by 4. We categorised a score <2 as "have not begun", 2-2.9 as "barely started", 3-3.9 as "underway" and a score of 4 as "completed [162].

3.4.4 Other covariates

We assessed the frequency of alcohol consumption with a scale from 1 to 5: "Never", "Once per month or less", "2-4 times per month", "2-3 times per week" and "4 or more times per week". Answers were dichotomized into "no" and "yes".

3.5 Clinical interviews

We assessed ethnicity, the possibility of pregnancy (exclusion criterion for DXA), acute and chronic diseases, use of medication and use of hormonal contraceptives through clinical interviews. Medication and diseases known to affect bone were dichotomized into yes and no. Diseases known to have a detrimental influence on bone are hypothyroidism, diabetes type 1, various eating disorders, celiac disease, and arthritis. Medication known to affect bone negatively are various types of corticosteroids, thyroid preparations and antiepileptic.

In girls, hormonal contraceptive use were categorized into "no hormonal contraceptive use", "estrogen and progestin" and progestin only"

3.6 Statistical analyses

In all three papers, analyses were performed sex stratified. Descriptive statistics were presented by means and standard deviations for continuous variables and by count and percentages for categorical variables. We compared and explored differences between participants and non-responders using Students t-test and chi-square testing. We used exact measurement dates to compute annual change to account for differences in time between measurements.

Significance level was set to $p<0.05$ in all analysis and all procedures were performed in SPSS. In paper I version 23 was used, paper II version 24 and paper III version 26. In paper II figures were made in RStudio (RStudio Team (2016). RStudio: Integrated Development for R. RStudio, Inc., Boston, MA URL http://www.rstudio.com/)

3.6.1 Paper I

Along with the description of changes in bone mineral levels over 2 years in Norwegian adolescents aged 15-17 years at baseline, the hypothesis of paper I were: (1) that participants remain in their original aBMD quartile between the ages of 15 and 19 years of age, and (2) that baseline predictors of positive deviation from tracking can be detected.

Differences in anthropometric- and DXA measures between TFF1 and TFF2 were tested using paired samples t-test, while dichotomous lifestyle factors were tested with McNemar's test. Mean absolute change (TFF_2 - TFF_1) and percentage change ((TFF2 − TFF1)/TFF1*100) for aBMD and BMC for each skeletal site were calculated. Participants were stratified by age (15, 16 and 17 years of age) and one-way ANOVA and multiple comparisons with Bonferroni post hoc test were used to examine differences in mean aBMD change between age groups. Calculations of individual age and sex-specific height-, weight-, FN-, TH- and TB aBMD and BMC z-scores (standard deviations away from the sample specific mean) were used to examine correlations between baseline and follow-up using Pearson's correlation coefficient. Partial correlation was applied to adjust for TFF1 height and weight as well as change in height and weight. Then we examined the proportions of participants that remained within aBMD and BMC z-scores quartiles, drifted upwards or drifted downwards between TFF1 and TFF2.

Then, an aBMD z-score change variable were computed (Z_2 - Z_1). Logistic regression were utilized to test whether baseline age, anthropometric traits (height, weight) and lifestyle factors (PA, alcohol consumption, smoke- and snuff use) were associated with positive deviation from tracking (z-score change > 0). The reference category was no change or downwards drift (z-score change ≤ 0). Odds ratios (ORs) with 95% confidence intervals (CI) for upwards drift during follow-up were calculated.

All models were adjusted for age, anthropometric measures, lifestyle variables, sexual maturation and time between measurements. The influence of other relevant confounders like baseline aBMD z-score, ethnicity, chronic disease and medication known to affect bone health bone and hormonal contraceptives use (girls) were explored and purposeful selection was used to select final model [163]. Relevant 2-way interactions were explored, identified and reported. We fitted models for FN, TH and TB separately, ran logistic regression diagnostics and assumptions were met.

3.6.2 Paper II

In the second paper the hypotheses were that baseline body weight/BMI and body weight/BMI changes over two years were associated with changes in aBMD and BMC parameters. Population characteristics were presented by BMI quartiles at baseline and we compared BMI quartile groups by using ANOVA with Bonferroni correction and χ^2 test. Welch's ANOVA with Games-Howell post-hoc procedure was used if equal variances assumption was violated. We computed annual bone- and anthropometric change variables to account for differences in time between baseline and follow-up measures in ANOVA analysis. To describe and explore crude impact of change in weight status on aBMD development, we stratified participants into quartiles of ΔBMI.

Associations between the exposure variables baseline BW, baseline BMI, Δ BW and Δ BMI and outcomes FN and TH ΔaBMD and ΔBMC during follow-up were further assessed by multiple linear regression analyses using the bone mineral follow-up score as outcome and baseline score as a covariate ($Y_2 = \beta 0 + \beta_1 Y_1 + \beta_2 X + \ldots$). Initially we conducted explorative univariate analysis. We then compared the results using change-score analysis ($Y_2 - Y_1 = \beta 0 + \beta_1 X$) and checking for consistency as baseline adjustments in change-score analysis may introduce bias [164, 165]. All adjusted models included anthropometric measures, time between measurements, pubertal maturation and perceived physical activity level. Other variables previously known to be of clinical importance (ethnicity, alcohol consumption,

smoking, snuff use, diagnosis known to affect bone, medication known to affect bone, hormonal contraceptives use) were then added as covariates using a backwards elimination strategy where p=0.10 were used as cut-off to enter or leave the model. Any covariate with $p \leq 0.10$ in a final model were included in all final models with the same outcome (ΔaBMD or ΔBMC). Based on this procedure alcohol consumption and diagnosis known to affect bone were excluded. We fitted separate models for baseline- and change exposure variables. Models with ΔBW were adjusted for Δheight. We checked for confounding and plausible 2-way interactions related to age, pubertal maturation and initial weight vs. weight change relationships. Because of statistical significance ($p<0.05$) we added interaction terms BW*menarche age in FN and BMI*menarche age in aBMD TH models in girls. In boys, a significant interaction between ΔBMI*BMI were included in FN models. Interactions were further explored and visualized by graphs.

Normal distribution, linearity, homogeneity and outliers were explored by residual analysis. In girls, two outliers were excluded in TH ΔaBMD: one in FN ΔaBMD and one in TH ΔBMC models. Furthermore, regression of baseline TB BMC on follow-up TB BMC lead to heteroscedasticity in residuals, and weighted least square regression approach were applied all TB ΔBMC models in girls.

3.6.3 Paper III

The hypothesis of inverse association between the exposure of snuff and smoking and the outcomes of change in aBMD between TFF1 and TFF2 in paper III where investigated by univariate and multiple linear regression models.

Population characteristics were presented by use of snuff status at baseline and groups were compared by using ANOVA with Bonferroni correction and χ^2 test. We used TFF2 score as outcome and included the TFF1 score as a covariate to estimate the predictive value of exposure on change ($Y2 = \beta 0 + \beta 1 Y1 + \beta 2 X_{Snuff} + \beta 3 \ldots$). We compared the results of the ANCOVA models using change-score analysis ($Y2-Y1 = \beta 0 + \beta 1 X_{snuff}$) and checking for consistency as baseline adjustments in change-score analysis may introduce bias in non-randomized settings [165]

Initially we conducted crude univariate models. Then potential confounders were added in the following way: “The anthropometric model” comprised the crude model plus age,

anthropometric baseline parameters and annual change in body weight and- height. In the full model, pubertal maturation and perceived baseline physical activity level were mandatory. In addition, variables previously known to be of clinical importance like ethnicity, alcohol consumption, smoking, diagnosis known to affect bone, medication known to affect bone and hormonal contraceptives use (all baseline measures) were then added as covariates using a backwards elimination strategy where p=0.10 were used as cut-off to enter or leave the model. All models were adjusted for time between measurements.

Normal distribution, linearity, homogeneity and outliers were explored by residual analysis. One outlier in both girls and boys were excluded. As in paper II, we used weighted least square regression approach to correct for the heteroscedastic pattern of residuals in the TB ΔBMC model in girls.

3.6.4 Handling missing and multiple imputation

We collected 688 repeated DXA measures at TFF1 and TFF2 and overall, missing variables were limited. However, because of late introduction of questions on sexual maturation (PDS-score) in TFF1 in boys 53 (17.9 %) participants were missing information on pubertal maturation. In all three papers multiple imputation of this variable were conducted to avoid losing a substantial proportion of the study sample, as recommended by Sterne [166]. In addition, a few missing variables of menarche age (n=7), physical activity (boys= 4, girls=1 missing) and use of snuff (n=2) were imputed. We assumed missing at random and we reported pooled estimates based on 20 repetitions [167]. Only exposures and covariates were imputed, not outcome variables. We performed sensitivity analyses and compared pooled estimates with complete cases in all three papers.

4 Results

4.1 Summary of paper I

The study sample were 358 girls and 296 boys and we measured femoral neck (FN), total hip (TH) and total body (TB) aBMD as g/cm² by DXA. aBMD increased significantly ($p<0.05$) at all skeletal sites in both sexes. Mean annual percentage increase for FN, TH and TB was 0.3, 0.5, 0.8 in girls and 1.5, 1.0 and 2.0 in boys, respectively ($p<0.05$).

There was a high degree of tracking of aBMD levels over two years in both sexes at aBMD-FN, TH and TB: Pearson's r = 0.960, 0.966 and 0.967 for girls and 0.937, 0.955 and 0.946 for boys, respectively ($p<0.001$). Age stratified coefficients showed somewhat weaker correlation at all sites for 15 year old boys (r=0.853 to 0.884). Overall, 73.0 to 79.6% of participants kept a stable position within quartile aBMD z-scores depending on site under consideration.

In girls, several lifestyle factors like snuff use, alcohol consumption and hormonal contraceptives use were associated with lower odds of positive deviation from tracking, whereas anthropometric measures appeared influential in boys ($p<0.05$). Baseline z-score was associated with lower odds of upwards drift in both sexes.

We concluded that our results support previous findings on aBMD accrual in adolescence. The fact that age is one of the strongest predictors of change is in indication that PBM has not been achieved. Tracking over two years of follow-up was strong. Baseline anthropometry and lifestyle factors appeared to alter tracking, but not consistently across sex and skeletal sites.

4.2 Summary of paper II

Paper II explored the associations of body weight (BW), body mass index (BMI) and changes (Δ) in weight status with adolescent bone accumulation in a sample of 651 adolescents, 355 girls and 296 boys, between 15 and 19 years of age.

Baseline BW and BMI were positively associated with ΔaBMD over two years of follow-up at all skeletal sites in boys, but not in girls ($p<0.05$). In boys, BW models per SD change in exposure adjusted for potential confounders showed following results: FN: $\beta=0.009$, $p=0.009$; TH: $\beta=0.009$, $p=0.002$; TB: $\beta=0.006$, $p=0.005$. ΔBW and ΔBMI predicted ΔaBMD and

ΔBMC in both sexes, with the exception of TB ΔaBMD in girls and the TH ΔaBMD model in boys.

Boys classified as underweight had significantly lower aBMD at baseline and this pattern persisted during two years of follow up. There were indications of threshold effects of BMI´s positive influence on bone as boys classified to be overweight had the higher mean aBMD than those classified as obese at both measurements.

Individuals who lost weight during follow-up demonstrated a slowed progression of aBMD accretion compared to those gaining weight, with statistically significant differences between BMI losers and BMI gainers in both ΔaBMD and ΔaBMC crude comparisons. The BMI-gaining boys had average TH aBMD increments around 1 %, while losers accumulated roughly half, and the differences were even clearer in ΔBMC. Loss of BW or reduction of BMI during two years was not associated with net loss of aBMD in the study population.

Although statistically significant, the magnitude of these changes in aBMD during follow-up was moderate and unlikely to have significant clinical implication on peak bone mass for adolescents with an adequate BW.

Our results indicate that underweight adolescent boys may benefit from a BMI increase. Particularly underweight individuals losing weight during this critical period of bone accretion could be at risk of a less than optimal peak bone mass acquisition, thus not achieving their full genetic potential for skeletal mass.

4.3 Summary of paper III

The main aim of this study was to explore associations between use of snuff and changes (Δ) in bone mineral parameters over 2 years in Norwegian adolescents aged 15-17 years at baseline. In addition, associations with smoking and double use were investigated. The study included the 349 girls and 281 boys that responded on questions on use of snuff and smoking at both TFF1 and TFF 2.

The study compared “non-users” with “users” of snuff, non-smokers with individuals that reported to smoke more than two cigarettes a week, and lastly, “non-users” with “double-users”.

In girls, comparisons of 244 “non-users” and 105 “users” of snuff showed no associations between use of snuff and ΔaBMD. In boys, 185 “non-users” and 96 “users” were compared and use of snuff was associated with reduced bone accretion in all ΔaBMD models. Sensitivity analysis with exclusion of “sometimes” users of snuff strengthened associations at femoral sites in girls and attenuated all associations in boys. In boys, adjustments for changes in anthropometric measures attenuated the associations between snuff and bone accretion.

In girls, no associations between ΔaBMD and smoking were found. In boys, only the TB ΔaBMD were significant, with a beta coefficient of -.011 and a p=.037. Moreover, in girls, “double users” was similar to smoking with a significant full model in FN ΔaBMC. In boys, nearly all models showed statistically significant differences, except the FN ΔaBMD model.

The study showed a negative association between use of snuff and ΔaBMD between “non-users” and “users” of snuff among boys and results indicate that snuff use and combined use of snuff and smoking in late adolescence could be detrimental to bone accretion and may be a signal of increased fracture risk in adult life.

5 Methodology discussion

5.1 Study design

The three papers in this thesis are based on data collected on two time points from a prospective population-based observational cohort study.

"a cohort study consists of a sample of individuals in a population followed up over time to observe changes in health status, to measure diseases incidence, and to examine associations between risk factors and health outcomes" [168].

Among the advantages of prospective cohort studies are the possibility of planning and implementing all the stages and events of the study from baseline to follow-up to fit the studies objectives [169]. Cohort data from prospective studies, if unbiased, reflect the "real life" cause-effect temporal sequence of events, and information collected from the same respondents sequentially over time is called longitudinal data. A two wave cohort designs is, in a strict sense, a longitudinal study. Such studies allow estimation of the amount of change in some parameters and measurements within a time interval. Although, studies of change are one of the cornerstones of research in health sciences, how to measure and represent change is a long-standing topic of debate. Nevertheless, there are serious limitations in the study of change with only two time points [170], and some authors even claim such studies do not qualify as longitudinal studies [171].

Two wave designs provide information on temporal order of events and are arguably more informative than cross-sectional study designs [171]. Temporality, i.e. the effect occurs after the cause, is regarded as the only essential Hill criterion for causality [172]. Two subsequent measurements on the same individual can detect within-person change and the major strength of this design is information on the amount of change with complete control for all time-stable confounders within the individual and thus, corresponds to a self-matched design [173]. However, as stated above it is argued that studies of change between only two time points do not estimate any causal or "longitudinal" effect, and "change on change" association models from baseline to follow-up are conceptually not estimating anything different from cross-sectional studies [173-175]. The only change that could be studied is linear and the question is whether it captures the true nature of the change [171].

Two wave design can be used in certain settings and address questions on average rate of change or comparing group means, not subject specific time trends and intra-individual change [176]. Exploring bone accretion in late adolescence involves change processes during growth and development. Developmental trajectories are continuously moving lines and it may be unnatural to explore a "chunk of time" between baseline and follow-up [177]. One major limitation with two measurements is that the shape of the trajectory is unknown.

Time is essential in cohort and follow-up studies and the primary aim is to study the influence of age [178]. However, there is a tremendous skeletal heterogeneity in adolescence. The timing of puberty and its inter-individual variability is essential in the comparison of change between groups [81]. Knowing that most modifiable determinants has their critical time-frames and is most influential during growth, individuals have to be aligned on biological age to compare like with like, e.g. in paper II, menarche age moderated the influence of baseline BMI on change in FN aBMD.

Another vital aspect is to determine the intervals of measurement. The spacing of observations should be in correspondence with theoretical model of change [171]. There is a considerable sex difference in the rate of change in bone properties and girls had significantly less change than the boys had, thus the follow-up time may be considered slightly less appropriate for girls given that some of the research questions in this thesis is related to prediction of change.

5.2 Internal validity

Internal validity refers to what extent results from a study are true, or valid, for the source population [168]. In other words, the extent to which observed effects in the dependent variable can be attributed to the independent variable [179]. Deviation from the truth may be due to random error, bias and/or confounding.

All measurements are subject to error and consists of two parts: the true score plus some error. Errors in epidemiological studies is not necessarily a problem if they are random. The best way to handle random error is by increasing the precision of measurements [180]. Large sample sizes will, according to the central limit theorem, approach the true parameter value

and reduce the impact of random error. Error can also be reduced by duration of study and frequency of measurements [181].

Bias can be defined as a systematic error in a conduct of a study and poses a threat to validity. There are no ways to "control for" bias in analysis stage, thus bias is an issue of study design and -planning [169, 182]. Awareness of bias helps researchers and readers to interpret findings more accurately. In cohort studies, bias can arise from two main sources. The first one is the approach chosen for selecting subjects and the second is the approach adopted for collecting or measuring data. These are termed as selection bias and information bias, respectively [183].

5.2.1 Selection bias and loss to follow-up

Selection bias in cohort studies occurs when a systematic error in the recruitment or retention of exposed or unexposed study subjects results in a tendency towards distorting the measure expressing the association between exposure and outcome [169].

Sampling of the Fit Futures cohort was based on geographic area and school. Although the majority of Norwegian adolescents attend upper-secondary school (93.4%) [157], of the potential 1301 individuals, 184 (14 %) were missing either because they dropped out of school, due of persistent disease or because we were unable to get in contact with them. This particular subgroup could differ substantially from the attending participants in various important exposure categories concerning lifestyle. Especially, those missed due to persistent diseases may have a different distribution of exposure and may well be individuals at higher risk of low bone mineral density and osteoporosis later in life. The invited cohort included 1117 participants and had an attendance rate of 92.9%. Studies show that non-responders are socially and biologically different from responders. Generally, non-responders tend to be poorer, younger, have lower socio-economic status and come from a less stable family and household [184]. Taken together, one of the strengths of the present studies is that the recruitment of the participants was population-based. The TFF1 survey collected data from more than 80 % of the background population and we consider the chances of selection bias due to non-response to be limited, but the possibility should not be totally ruled out.

Changes in aBMD and its predictors is the topic of interest in this thesis. Thus, the criterion for inclusion in all the three studies was participation in both TFF1 and TFF2. Although prospective study designs make selection bias less probable than other designs, cohort studies are prone to dropouts [182]. Loss to follow up is a threat to validity if dropout rates differ between study groups; or if drop-outs are different from those who do not drop out. A low follow-up rate increases both the risk of a type II error when evaluating the outcome and the risk of a non-representative cohort [185].

The distinction between non-responders and loss to follow-up is that information about dropout attributes is available for comparisons with the initial study population. In our studies, a large proportion of dropouts were boys. Not counting participants above 17 years of age at baseline, 111 girls and 196 boys were lost to follow-up. Among girls, TFF2 dropouts (<17 years of age at baseline) had statistically significant higher BMI than responders. This could indicate that our study population in paper II had a slightly different body composition compared to the reference population. Previous studies have shown associations between BMI and bone mineral density. Since BMI is positively associated with BMD, an underestimation of the association would be expected.

Dropouts had significantly higher prevalence of daily smoking, snuff use and alcohol consumption (girls only) as well. The differences in use of tobacco among both girls and boys may also have influenced results in paper III. A higher proportion of smokers and users of snuff in background population will lead to an underestimation of the associations in the study population.

There are numerous reasons for loss of follow-up: difficulties locating participants, migration, relocation, change of school and refusals to further participation [184]. It is well recognized that healthier people and those who are more concerned about their health condition are more motivated, and likely, to participate in epidemiological and clinical surveys [186]. The best way to eliminate the impact of bias to loss to follow-up is to keep losses to an absolute minimum [187]. A great deal of efforts were put into retaining TFF1 participants, e.g. free transport for relocated individuals to TFF2, even by plane if necessary. Studies with less than 70 - 80 % follow-up should be viewed cautiously and with skepticism regarding this kind of bias [180]. Given that TFF2 includes only 66 % of the original baseline cohort, we ought to be aware of the possibility that follow-up loss could correlate with both exposure and

outcome. Studies indicate that loss to follow-up not necessarily cause bias and undermine study results. Minimizing loss, however, increases the precision of the estimates [168, 188, 189]. The high attendance at baseline gave the opportunity to compare the study population with a relatively representative background population. Nevertheless, the possibility of our study sample being a healthy subpopulation remains.

5.2.2 Information bias and misclassification

Information bias refers to systematic errors in the information collected from the study participants. Measurement error and poor quality and accuracy of information carries a risk of information bias [168]. A main source of bias in cohort studies relies on the degree of accuracy in which participants are classified with respect of exposure status [187]. Misclassification of exposure status might be a source of information bias and occurs when participants are classified into an incorrect category. The misclassification can be non-differential or differential. Non-differential misclassification occurs when the probability of being misclassified into categories of any variable is the same for all study participants, while differential misclassification occurs when the probability is different across groups of the study participant. Particularly differential misclassification is a concern in research as it may distort effect estimates in both directions, while non-differential misclassification usually leads to an attenuation of association between exposure and outcome [186].

In TFF, a major part of lifestyle variables was collected using computer based self-administered questionnaires. Subjective assessments and questionnaires may obtain inaccurate and insufficient information about lifestyle and other determinants of change in bone mineral density. The questionnaire contained some questions on previous behavior (e.g. average during last year) and such information are prone recall bias. Participants may give incorrect information, consciously or not. They could also be putting low effort into interpreting and answering questions adequately and might avoid extreme values or exaggerate answers for different reasons [169].

Item non-response refers to missing answers to questions or incomplete examinations [168]. The TFF questionnaires were relatively large which may lead participants not to respond to all questions with the same awareness. During the “TFF-day” at the hospital, participants were given time to answer the questionnaire between clinical examinations and various

measurements to minimize this kind of bias. Item non-response were otherwise handled by multiple imputation approach in the analysis process (see 5.2.8)

5.2.3 Validity of use of tobacco assessment

When asking sensitive questions on habits like use of snuff and smoking there is a risk of social desirability bias and under-reporting. However, some studies indicate that self-reported smoking is fairly valid in adolescence [190], and the relatively wide (and increasing) scale of snuff use among adolescents may have made this behavior more socially acceptable. Prior to, and during the TFF surveys confidentiality was stressed and no names were attached to the questionnaires, which could contribute to truthful reporting.

There are challenges, however, regarding quantifying and setting the thresholds for tobacco exposures and the questions used in TFF on tobacco use are not validated with respect to an adolescent population. Adolescence is a time of change and experimentation, and as discussed in paper III, responses on use of snuff were unstable, and responses were not always coherent. A lot of "sometimes" users of snuff at TFF1 ended up in the daily category in TFF2 and it is a challenge to interpret individuals responding "sometimes" use of snuff at TFF1 and "No, never" two years later at TFF2. These are indications that the two responses two years apart may have shortcomings in determining exposure and the probability of misclassification is present. Participants in the "sometimes" reporting "one or less" portion of snuff a week could approach the "never-users" group and the "more than 10" portions weekly may exceed "daily-users" in exposure. An observed clear tendency of increased frequency in portions used a week in the baseline "sometimes" group during follow-up may have influenced the degree of accuracy in which the participants have been classified with respect to their exposure status.

We consider the possibility of differential misclassification between "non-users" and "users" of snuff to be negligible. However, the possibility of participants denying use of snuff or smoking, for some reason, cannot be ruled out. In such cases the associations in paper III would be underestimated. The likelihood of misclassification increases between "sometimes" and "daily" users of snuff, but in such cases we consider those to be primarily non-differential.

5.2.4 Validity of body weight and BMI

Clinical anthropometric measurements have generally high validity, but it relies on standardized training, standard operating procedures, robust equipment and measurement resampling [191]. One of the strengths of the TFF studies were that clinical data e.g. anthropometric data were measured directly by research technicians according to standard protocol.

However, biological variability is an inherent part of nature and natural variations can be misinterpreted as associations. The rate of change in physiological processes may fluctuate due to many factors (e.g time), and the variance may be high because of biological factors. In paper II, body weight may vary during different seasons, as the follow-up time varied from 1.50 and 2.67 years. Nevertheless, we consider such variation to random and the risk of bias in body weight, height and BMI to be negligible.

5.2.5 Validity of physical activity assessment

Physical activity is regarded as a confounding factor[1] in all three papers. The four-level questionnaire used to assess physical activity, was initially introduced by Saltin and Grimby in 1968. The questionnaire is widely used to assess physical activity in population-based studies, especially in the Nordic counties. Since the beginning, it has been used by more than 600 000 subjects. Over the course of years, minor modifications have been made, such as changing practical examples of activities to illustrate the levels of physical activity. The modernized Saltin-Grimby Physical Activity Level Scale (SGPALS) was validated in the adult population, and recommended as a useful tool for routine risk assessment [192, 193]. The questionnaire is validated in the Tromsø Study as well [194]. Self-reported physical activity have some disadvantages though, and tends to be over-reported, due to “desirable” reporting and this may be exaggerated by the fact that TFF were advertised as a "health survey" [195, 196]. Furthermore, participants were asked to give an average for the last year and questions about the past are prone to recall bias. Taken together, several studies show that

[1] See 5.2.11 for definition

questionnaires yield reasonably valid measures for epidemiological research [197, 198] and we believe the assessment of the physical activity levels of the TFF participant to be valid.

5.2.6 Validity of pubertal maturation status

The influence of puberty on the skeleton must be considered when evaluating change in aBMD [103]. Pubertal stages are closely linked to linear growth and bone accrual, thus the precision of pubertal maturation estimation is a key to the validity of the inferences made in all three studies. In the analysis process, stratification of girls and boys were used to deal with the maturational gender differences.

Tanner staging from physical examination is regarded as the gold standard for assessment of pubertal maturation [199]. Due to feasibility issues, this approach was not included during planning of TFF study. Pubertal status was self-reported by the participants in TFF, which may induce some bias. Questions on menarche age and secondary sexual characteristics may be a sensitive subject and therefore answered inaccurately or not at all. However, the proportion of missing puberty data in girls was three percent, this is not notably higher than other lifestyle variables (2%), and we consider these to be missing at random.

Menarche is a hallmark female maturational event and the age of onset is used as a proxy for sexual maturation in girls. The question of year and month of menarche age has good recall accuracy [200].

In boys, the PDS-score was utilized as maturation assessment. This questionnaire has shown to be a non-invasive alternative to Tanner stages, although its precision has been questioned. Previous studies have compared physical examination to self-report and the results are diverse. Two studies among Chinese and Canadian adolescents boys found the agreement to be strong, with a Cohen's kappa coefficient between 0.58 and 0.80, while one study with black, multiethnic South African youth concluded with a low to fair agreement of 0.26 in boys [201]. The late introduction of the PDS item in the TFF1 survey resulted in 23 % missing values in boys. This is considered as a limitation in our studies, even though multiple imputation techniques were applied.

Self-reported PDS-score may be used when precise agreement is unnecessary [202]. In paper III, additional adjustments for changes in height and weight were necessary, since it turned

out to be a major confounder in the use of snuff and bone accretion relationship, indicating that PDS-score may be too rough an estimate of maturation in studies during growth.

5.2.7 Validity of other covariates

Alcohol consumption and contraceptives use are prone to recall bias and underreporting in line with tobacco, as discussed above. A limited number of trained study nurses conducted the clinical interviews and we consider the information on medication and diseases that could affect bone to be valid.

5.2.8 Validity of multiple imputation

Multiple imputation is an approach to deal the problem of missing data and item non-response. Valid inferences after this technique are obtained because the approach are averaging over the distribution of the missing data given the observed data [166]. *"The imputation procedure must fully account for all uncertainty in predicting the missing values by injecting appropriate variability into the multiple imputed values; we can never know the true values of the missing data"* [166]. The predictive distribution of the observed data is used to sample the imputed values. Outcome variables, exposure of interest and covariates in the full model were used in the prediction. First, multiple copies of the dataset were created where the missing data are replaced by imputed values. The number of copies varies with the degree of missing information and tolerance of power falloff, and in our study 20 imputations seemed sufficient [167]. Next, models were fitted for the 20 imputed datasets. Estimations varied within each dataset because of the variation introduced by the imputed values and we reported the pooled values of these estimated associations.

5.2.9 Validity of DXA measurements

Generally, DXA scans has excellent precision and are considered to be one of the most precise methods of measuring bone mineral density and the reproducibility is far better for aBMD scans than for most laboratory tests [203]. However, aBMD measures are accompanied with measurement error and any interpretation of change requires knowledge of measurement precision [204]. Repeated measurement of the same subject will vary around the true value because of measurement error. This could be because of natural variation within the individual or variation in the measurement process, or both [205]. With only two repeated

measurements there is a modest, but basic, concern to estimate true change of aBMD based on observed change with proper regard for reliability of the measurement [176].

DXA measures are affected by both precision and accuracy errors. Accuracy refers to whether the measured value reflects the true (or actual) value of the object measured. The typical accuracy error of a DXA scan is 3-9 % depending on measured site [206]. This is below the WHO ten percent criteria for diagnostic tests. These accuracy errors are mainly random errors caused by heterogeneous lean-, and fat mass distribution [207].

In a baseline to follow-up study trying to detect small percentages of bone mineral gain, precision is the major concern in order to separate random error from true biological change. The latter is only detectable on an individual level if change over time exceeds measurement error. In the present thesis, this applies particularly for the girls. Paper I revealed gender differences in accretion in late adolescence. Girls seemed to approach the end of longitudinal growth, while the boys had not reached their final stature. PBM is not reached at the cessation of longitudinal growth, but the bone accumulation will naturally slow down.

To keep errors to a minimum, we used the same instrument and software at baseline and follow-up and followed the manufacturer's algorithm for calibration as current recommendations for longitudinal scans by ISCD. Precision depends on quality assurance of the performance of the DXA instrument. Phantom scans were performed each morning throughout both surveys and no drift were detected. Another requirement is exact repositioning of subjects and introduction of most errors are due to operators and subjects' variability rather than machine performance. [208]. Trained professional staff followed a rigid protocol with rigorous attention to detail in positioning. However, because of the high number of participants in the study, different technicians operated the DXA from day to day, which could influence precision, however these errors were most likely random. The participants were asked to remove any jewelry, wear light clothing without zippers or metal closures. Subsequently, images were re-analyzed in a scan-quality control. Artifacts, metal objects and errors in the automatic image analysis were eliminated if necessary.

Even if measures were taken to keep errors to a minimum, interpretation of the results of the papers in this thesis must take into consideration the precision of the DXA measurements. The aBMD values of an individual must change 2.77 times the coefficient of variation of the

instrument for real change detection [209]. No exclusive precision study was conducted prior to the TFF survey, but the densitometer CV ([SD/mean] x 100) has in a previous study been estimated to 1.14 % at the total hip and 1.72 % at FN measured in vivo [210], which is in accordance with reports from other devices [28]. These CVs were elicited from an adult sample, and there are reports of more favorable variability when measuring young healthy subjects [211]. This may be partly because of easier positioning [212]. The CVs gives a least significant change for the TH =3.157% and the FN =4.764%. Thus, the significance of the observed changes at an individual level is debatable. Nevertheless, the measurement errors are assumed to be random with a net effect of zero and the reproducibility of BMD measurement by DXA expressed by different means is good at a group level [203]. Our sample size is relatively large, we see clear consistent patterns of change, findings are consistent with previous research and by epidemiologic standards, bias produced by measurement error is relatively minor due to the high precision of DXA scans [181].

The major limitation of DXA-scans are that it is a two-dimensional projection image and it is affected by bone size. To handle the size-related artifact, all analysis were conducted sex stratified and all statistical models were controlled for height or change in height, if appropriate. These adjustments served to identify changes in bone traits that are independent of changes in stature. The relation between aBMD and true volumetric density is non-linear. Paradoxically, this error can improve the value of BMD as a predictor of fracture risk, since bone size is also a determinant of skeletal strength [40].

The second limitation is that the body consists of 3 types of tissues bone, lean and fat even though DXA is only able to distinguish between two different materials [206]. Shape, body habitus and changes in body composition may affect DXA measurements. The impact of thickness of body tissue overlaying the measured area could be a concern in longitudinal studies of the influence and changes of body weight [213]. However, this implies especially to lateral scans not performed in this study [214, 215]. Yu et al. found that changes less than 6 kg fat layering did not affected DXA results [216]. In paper II roughly 30 % of the participants gained or lost > 6 kg between measurements. It is worth noting that if an individual changes > 6 kg the amount of fat layering lost or gained in a single skeletal site would be less, and the risk of bias in this study population is minimal.

5.2.10 Statistical modelling

Different variations of change from baseline also known as “delta”, “difference scores” and “gain scores”, were utilized in all three papers. Change-score is an elementary measure and a summary of the observed within-individual change in the response [217]. However, the issue of comparing the amount of change between two time points in non-randomized contexts is complex and decision making on available statistical approaches is challenging [218]. There have been great controversies on the use of such methods. Numerous caveats have been identified and described: unreliability [219], ANCOVA vs change from baseline in non-randomized studies [165, 170, 220], mathematical coupling [221], regression to the mean (RTM) [222], Lords Paradox [223, 224] and baseline adjustments [225].

However, change-scores is widely used, especially in the field of psychology and the approach has its supporters [173, 176, 226, 227]. Early reliability concerns about change-scores are no longer seen as obstacles and research has shown that these concerns tend to apply to unusual situations and that change-scores can be reliable in many typical research settings [220].

There are several statistical methods of comparing groups concerning change, but **change-scores** and **analysis of covariance (ANCOVA**) are frequently used. The simplicity of change-scores is one of its advantages. It is intuitive and easy to understand [170]. An outcome score obtained by subtracting initial value from follow-up measure for each individual $(Y_2\text{-}Y_1) = \beta 0 + \beta 1 X_{Exposure} + \beta 2 X_{Covatiate}$. Where Y is the dependent and X’s are the independent variables. When calculating a change-score participants are essentially used as their own control. However, change-scores do not differentiate between participants high and low initial values at baseline [228].

One of the problems inherent in repeated data is that they are correlated by design [229]. Measures of aBMD within subjects at baseline and follow-up are naturally correlated, and this violates assumptions of independent data in some statistical approaches, like regression analyses (if tempted to analyze all individual relationships at both time points at once). Simplification of the statistic to a single number for each subject by taking the difference of two time points avoids dealing with the longitudinal correlation. Criticism of this approach points out that this reduces the dimensions of the data [230].

ANCOVA was used in papers II and III and is a special case of a general linear model and adjusts each subject's follow-up measurement according to his or her baseline measurement. The approach is sometimes referred to as a model of residualized change, offers high statistical power and can be summarized using the equation: $Y_2 = \beta0 + \beta1Y_1 + \beta2X_{Exposure} + \beta3X_{Covatiate}\ldots$ [222]. The main interest is in the potential influence of some grouping variable on a bone parameter at TFF2, but we want to make sure to include the initial value at TFF1 in the model to control for baseline levels. Change-scores and ANCOVA asks different questions. Change-score asks which group changes most, while ANCOVA answers a conditional question: "If the groups had come from a population with the same baseline level, which would have increased or decreased more?" [218, 225]. One approach estimates the total effect (of exposure on bone accrual) while the other estimates the direct effect, adjusting for the initial bone trait level [231].

An alternative approach is ANCOVA with difference scores: $(Y_2-Y_1) = \beta0 + \beta1Y_1 + \beta2X_{Exposure} + \beta3X_{Covatiate}$. This model includes the change-score as the dependent variable and baseline measurement as an independent variable. If baseline measures have to be taken into account anyway and added as a predictor in the equation, it makes no essential difference whether the outcome is the change- or follow-up score because the results will be identical due to basic algebra [220]. However, ANCOVA with follow-up score were preferred in paper II because the residual plots using ANCOVA with difference scores turned out heteroscedastic in most models. The ANCOVA with difference scores approach was indirectly used in the logistic regression models paper I as positive or negative deviation from tracking were based on the follow-up z-scores minus baseline z-scores and then adjusted for initial z-scores.

Regressing the change-score on initial measure have some inherent bias [205]. Two methodological concerns have been raised, **mathematical coupling** and **regression to the mean (RTM)**. Mathematical coupling occurs when a variable contains the whole or parts of another and then analyzed by regression or correlation. The error term of the baseline measure occurs on both sides of the equation making the test of the relationship between initial and change-score biased. The relationship tend to be negative and consequently, the statistical procedure of testing the null hypothesis might then no longer be appropriate and the results must be interpreted with caution [232].

RTM is a statistical phenomenon caused by random error of measurement and/or to physiological variation that can complicate group comparisons [188]. It occurs with any repeated measurement that fluctuates within an individual or a population. Individuals with scores far from the mean value of the distribution (extreme measures) in TFF1 will tend to be closer to the average value on second measurements in TFF2. Baseline values are negatively associated with change-score because the low value at baseline will get a higher follow-up score and higher baseline value will be closer to the mean at the follow up measurement. RTM a common phenomenon because error free measurements is rare [222].

It is in scenarios where the correlations between Y1 and Y2 is low the effects of both mathematical coupling and regression to the mean is greatest [233]. When assessing the degree of tracking in paper I we showed that the correlations between baseline and follow- up bone traits were high (r=0.93-0.96), so the impact of these statistical phenomenons should be recognized, but not considered critical to the studies´ validity.

The rationale behind taking into account the starting situation in studies of change is debated. In studies of growth and development it is not always apparent when the trajectory starts and the definition of exposure itself may be unclear [234, 235]. Baseline may not even be the appropriate term in TFF because there is only one measurement. In paper II, there is an issue of temporal order of events. The baseline differences in bone outcomes according to body weight and BMI categories were apparent. Looking at the hypothesized causal relationship, the baseline bone mineral status may be considered as a mediator between body weight (and BMI) and final bone mineral status. This may be explained by of the substantial impact of body weight (and size) on bone accrual early in life and especially in puberty [236], and as expected, body height and -weight had already influenced our participants skeletons.

Baseline adjustments in observational studies comparing preexisting or naturally occurring groups may inflate the regression coefficients and make change-score and ANCOVA approaches reach contradictory conclusions – a bias known as "**Lord's Paradox**". The main concern is that this mediation could produce bias when baseline outcome parameters are included in ANCOVA models. If baseline outcome and exposure of interest are strongly associated, biases induced by baseline adjustment can be quite large [164]. In paper II, this applies especially to the TB BMC and body weight/BMI models as the total content of minerals in a skeleton is closely related to the size of an individual.

Randomization is not present in cohort studies and there is no reason to expect that the baseline means for the study groups should be equal. That concerns both measured and unmeasured exposures. However, the major consideration in change-score analysis is baseline differences in the predictor of interest [165]. Lords paradox is especially common in fields were designed experiments and trials are not possible, e.g. to randomly assign adolescents that takes recreational drugs is not possible.

In paper III the relationships and the initial bone scores between comparison groups were slightly different. The mean onset of snuff were around just above 14 years of age and bone outcomes were relatively equal at baseline, maybe because the hypothesized influence of snuff had not been established.

Ideally, ANCOVA should be used to partial out variance of covariates that have a high correlation with the outcome variable and no relationship to exposition [225]. If this approach is used, it is essential to note that measurement error always produces under-adjustment, which could results in a directional bias. The rule governing this bias is that "consistent" differences is magnified. I.e. groups with the higher baseline increasing more or groups with the lower baseline decreasing more. On the other hand ANCOVA also masks 'inconsistent' differences [225]. The rate of type 1 errors increases with both measurement error and baseline differences [225]. In paper II, a significant baseline difference were observed and this directional bias has to be considered when concluding that baseline body weight is positively associated with change in body weight. We handled this dilemma according to advice by van Breukelen [165], compared the results from the two approaches, and advocated caution in the interpretation of result with discrepancies.

Each approach has its strengths and limitations and the decision on which one to use depends on what assumptions you want to make. Assumption of change-score is that change is independent of group, (i.e in a RCT the "never-users" group will not change). The assumptions of ANCOVA is that change is a linear function of the baseline and that this holds for all subjects in the group. If the study sample comes from one population, the model considers it unusual to have group differences at baseline and expects both groups to regress to the mean. If the group means stay equal across time this may be considered as an "effect" in the model. Change-scores and t-test does not make this assumption. However, these assumptions are untestable in an observational study [165].

In a wider perspective, it is open to discussion which approach gives the correct “real life” answer. It would be expected that skeletal changes are greater in larger individuals than in smaller ones and when assuming equal baseline levels, "*…then the adjustment is comparing entities that not only do not exist, but (probably) cannot exist*" [237].

To sum up, some assumptions of these models may lack theoretical justification [238]. Two-wave change-score analysis is not exactly wrong, but it is not quite right either. More suboptimal, as stated by Norman & Streiner [239]. However, both cross-sectional studies and longitudinal studies are prone to the same fallibility in potential errors [240] and change-score could be considered as an unbiased measure of change (a process) between two separate status measures (not a process) [241]. The inherent problems in repeated data and their relationship with random and measurement error makes it crucial to maximize reliability at both baseline and follow-up to make the change-score valid [228]. It is not always about reliability of change-scores, but also whether they are more reliable than viable alternatives [242].

5.2.11 Confounding and interaction

In cohort studies, **confounding** is a threat to the internal validity and can be described as: *"...a situation in which a non-causal association between a given exposure and an outcome is observed as a result of the influence of a third variable (or group of variables)*" [169, p. 153]. The confounder must be causally associated with outcome and non-causally or causally associated with exposure. The variable should not be in the causal pathway between exposure and outcome, and must be unequally distributed between comparison groups to have an impact on the association [168].

Analysis of associations relies on the assumption that outcome will be different if the same individuals are exposed or not. Individuals in a population cannot be exposed and unexposed at the same time. The solution to this problem often referred to as the counterfactual model, where a substitute population is used as a comparison group. This introduces confounding and the principle of comparing like-with-like in relation to confounding is vital [168].

Existing knowledge and previous research on skeletal and bone health are important to identify and choose possible confounders, and to avoid data-driven models. There are

different strategies to control for confounding. We primarily used restriction, stratification and statistical modeling to cope with confounding. We used a restriction of age less than 18 years, with the disadvantage of a smaller sample size [180]. All analyses in this thesis are done sex stratified because of the known biological differences of bone development between boys and girls in adolescence. Adjustments through statistical models will reduce the probability of confounding. However, the probability of residual confounding is present.

The measurements in TFF do not cover all differences in compared groups. Imprecision- or lack of measurements leads to residual confounding and candidates for potential unmeasured confounders in this study is calcium intake, vitamin D and hormonal factors. Nutritional data in TFF has been criticized for being incomplete. This is a concern when it is likely that nutrition is a part of the causal network and is considered an important determinant of peak bone mass. However, validated nutritional data were not prioritized because it would occupy a large proportion of the selected questionnaires and would be time consuming to answer for the participants.

Another matter is the choice of baseline vs. change in exposure variables. In paper II change in body weight and BMI were investigated. Changes in anthropometry is closely linked to bone. The main problem is the lack of information on when the changes took place. The age group represented by TFF1 (15 to17 years of age) dropout from organized sports is conceivable. Changes in physical activity during follow-up, e.g. resigning from participation in hard training or sports competitions right after TFF1 survey would potentially have an influence, even if the parameter is only a covariate.

Interactions (or association modifying) are a major concern in the multiple linear regression analysis process and may, if not dealt with appropriately, lead to erroneous interpretations. Statistical interaction arises when considering the relationships among three or more variables, and the association between outcome and one exposure is moderated depending on levels of a second exposure [169]. The strategy of detecting interactions in our studies were based on a combination of a priori knowledge and statistical significance, and the risk of undetected (clinically interesting) interactions cannot be ruled out. In paper I, age moderated the influence of body weight on deviation from tracking, but this interaction attenuated when associations between body weight and absolute change in aBMD where explored in paper II. On the other hand, a statistically significant interaction between baseline body weight and

change in body weight were explored. In addition, menarche age moderated the relationship between BMI and change in aBMD. In paper III, age*double use and age*snuff interactions in the ΔaBMD TB models were identified and reported in girls and boys, respectively.

5.3 External validity

External validity refers to generalizability outside and beyond the source population, i.e. other youth populations. To what extent observed associations can be applied to other populations relies a great deal on the internal validity of a study. Generalizability depends on similarity in characteristics between the study population and the population you want to compare with. [180].

Characteristics of the study participants in the three papers showed similarities compared to those in other Norwegian studies, e.g. the Young- HUNT study from middle parts of Norway and Bergen Growth Study from the western parts of the country. However, the prevalence of overweight and obesity among children and adolescents have been slightly higher in Northern parts of Norway than in other comparable cohorts [243] [244] [245]. The physical activity levels were also a bit higher in TFF than reported from the Young- HUNT cohort [246]. Associations between body weight, physical activity and bone traits indicate that there may be regional differences in aBMD in Norway and the results may not be valid for all Norwegian adolescents. The prevalence of smokers and users of snuff in TFF is similar to reports from the Norwegian Health Institute and Northern Norway is comparable with other regions in Norway [144].

The TFF study sample is a convenience sample. The general criticism of such samples is that it may not be representative of the entire population. However, school sampling may be representative of certain age groups [168]. Most Norwegian adolescents attend upper secondary school and the attendance rate were high at TFF1. Nevertheless, loss to follow-up (~39 %) and the high proportion of white ethnicity complicates extrapolations to the entire Norwegian youth population. Furthermore, reservations must be made because the study population is from two municipalities of Northern Norway and differences in topography, exposure to sun etc. may influence the generalizability.

The physiological aspects of paper II and paper III may be more applicable to other populations. The biological mechanisms behind the influence of body weight and tobacco on bone are likely to apply for other populations as well. To sum up, caution of generalizing is advised, but we consider the TFF cohort to be fairly representable for Norwegian adolescents between the ages of 15 to 19 years of age.

6 Result discussion

In this thesis, we have explored how lifestyle influence bone accretion in a Norwegian population in late adolescence. The follow-up design with two measurement points gives a sequential order of event, but all results in this thesis derives from models that are used to show statistical associations, not to infer causation [173]. Nevertheless, the discussion of main findings will be based on some of the guidelines Bradford Hills proposed as aid for the researcher to judge to what extent a causal interpretation is reasonable when a statistical association is found in an epidemiological study [247]. Issues like consistency of results i.e., previous research, temporality, strength of relationships, biological plausibility of results and biological gradient/dose-response will be discussed if suitable. The influence of lifestyle factors on changes in aBMD and BMC links the three papers together. Body weight, BMI, Δbody weight, ΔBMI, use of snuff and smoking figures as both exposure of interest and/or covariates in this thesis. If appropriate, associations were controlled for potential confounders like physical activity, alcohol consumption and contraceptives use (girls). The discussion of results is organized according to the aims of the three papers: Description of change in bone traits, degree of tracking during two years of follow-up, influence of body weight/BMI and influence of use of snuff and smoking on bone accretion.

6.1 Change in aBMD and BMC during two years in late adolescence

In order to explore determinants of change bone traits in the TFF cohort we first had to establish that change occurred during follow-up, and to what degree. Paper I was mainly descriptive and aimed to capture the dynamics of bone traits in late adolescence. aBMD increased significantly ($p < 0.05$) at all skeletal sites in both sexes, however changes were approximately double in boys compared to girls, e.g. ~4 % vs. 1.6 % in TB during two years of follow-up. Based on the ~2 year difference in maturation, and prior research, girls were expected to approach PBM at femoral sites and mean changes over two years at the FN were down to 0.6 % compared to 3 % among boys. The bone accrual rate had an inverse relationship with age and the rate decreased consistently at all skeletal sites, in both sexes, but boys did not appear to be approaching cessation of longitudinal growth to the same degree as the girls did. As mentioned before, the intra-individual variability is not likely to be detect in two-wave studies, but by comparing the accretion at femoral sites in girls at different chronological ages, there were indications of accretion levelling off and reaching a plateau in

the oldest subgroups. However, the sample size became limited when stratifying by age, reflected by the wide confidence intervals.

A surprise finding in this paper was the decrease in FN aBMD for girls between 17 and 19 years of age. However, Berger et al. reported similar findings with an average decrease of aBMD in girls around 20 years of age until stabilization and consolidation. Disentangling this result by exploring BMC and BA showed that BA increased while the BMC measurement had stabilized, which is in line with the notion that adolescent skeletal growth is not necessarily density, but mostly due increased bone size [84]. Furthermore, Bachrach et al. reported cessation of accumulation even earlier than our findings. In girls, gains in aBMD leveled off in total hip, spine, and whole body already at the age of 14.1, 15.7, and 16.4, respectively. Boys tended to reach plateau at the age of 15.7 in total hip and 17.7 in spine and whole body [248]. However, the observed decrease in our study may also be a chance finding due to low sample size or measurement error.

If PBM is a major determinant of future risk of osteoporosis and fractures, the timing of PBM is of importance because it provides knowledge of when the "window of opportunity" is open, and when it is about to close. There have been some controversy around the timing of PBM due to differences in statistical approaches and parameter used, but longitudinal studies with appropriate maturational data seems to have brought consensus to the matter of age estimation and the sex-, ethnicity- and skeletal variations that exist. PBM is site specific and femoral sites reaches its peak at 16 to 19 years of age, while the lumbar spine aBMD peaks more than a decade later, between 33 and 40 years of age [10, 248, 249]. Studies with repeated measures are preferred in description of PBM because they capture the process of bone accretion. The fact that age was a strong predictor of change in aBMD for both girls and boys in this study population indicate that PBM is not yet reached.

6.2 Tracking of bone mineral density

One of the hypothesis behind the concept of PBM is that individuals with low PBM could suffer high fracture risk later in life [85]. However, high PBM counteracts age-related inevitable bone loss only if bone trajectories tracks throughout life. We found a high degree of tracking using study sample based z-scores. Pearson's correlation coefficient between TFF1 and TFF2 aBMD FN, TH, and TB, were 0.960, 0.966, and 0.967 for girls and 0.937, 0.955,

and 0.946 for boys, respectively. We detected some changes in the rank order within the distribution, but with the relatively short follow-up time of two years, extensive drift between quartiles was not expected. Stratification by age showed that the correlation were lowest among the youngest participants.

Several studies have explored tracking with various ages and follow-up time, and most of them confirm that bone mineral levels track strongly with a correlation coefficient between 0.5 and 0.9 [93-95, 250]. Follow-up during peak height velocity is expected to show reduced correlation, and lower correlations in younger children than in older have been reported [93]. In our cohort, aBMD tracking for boys became successively stronger as annual height change reduced gradually between 15 and 17 years of age at baseline, indicating this link between statural growth and aBMD tracking.

There is, however, a paucity in tracking data from adolescence into adulthood and there are several well-controlled clinical studies that indicate that acquisition of a high PBM during childhood and adolescence will have only transient effects, and advantages will diminish if osteogenic determinants (e.g. physical activity) are not sustained [12, 97]. Taking into consideration the understanding of the skeleton as a homeostatic that is constantly sculpting bone through adaptive processes to meet the mechanical requirements (as elaborated above), this transient effect is plausible. On the other hand, there is indications of geometric and structural changes accompanying bone accrual, e.g. the racquet arm of tennis players, which has a sustained impact on bone strength [77].

The key issue seems to be “tracking throughout life”. Osteoporosis has been called a pediatric disease with geriatric consequences because of its complex and life-course perspective etiology [251]. The hypothesized cause of inadequate PBM and supposed effect of increased fracture risk is virtually a lifetime apart, and the feasibility of lifelong longitudinal studies is low.

Some tracking studies have also looked at factors that predict deviation from tracking. We found that baseline aBMD z-score was the only consistent predictor of deviation from tracking in both girls and boys. Among boys, baseline body weight tended to be associated with upwards drift in aBMD z-score at femoral sites. Among girls, lifestyle factors such as physical activity snuff use and consumption of alcohol appeared important, but not

persistently across skeletal sites. The lack of consistency related to skeletal sites and sex is a concern related to the validity of our findings and calls for further investigation and clarification.

The use of z-scores and deviation from tracking (paper I) provides a slightly different perspective than exploring absolute numbers of bone accrual (paper II and III). The deviation from tracking shows how accrual changes in relation to the study population distribution as a whole, and if various strong determinants (e.g. physical activity, use of snuff or smoking) also tracks during follow-up, no deviation from tracking would be expected because we only adjusted for baseline parameters.

6.3 Association between body weight/BMI and change in BMD/BMC

After investigating and describing change in bone traits over two years in paper I, the natural next step was to explore factors that predict this change. Obesity and overweight in childhood and adolescents are a growing concern worldwide with rising prevalence during the past decades worldwide [107] and in Norway [106]. In the present study, more than one of five adolescents were classified as overweight or obese at baseline and the prevalence increased during follow-up in both girls and boys. In paper II, we excluded three participants from the initial paper I with various missing outcome variables and included 355 girls and 296 boys in the analyses.

The “osteogenic” influence of body weight and BMI in the adult and older population is well established. A low body weight in older individuals is a risk factor of fracture and maintenance of-, or body weight gain could have a protective effect by preventing bone loss. The hypothesized mechanisms behind this is extensively discussed in paper II and section 1.4.1. There is, however, conflicting evidence whether excess body weight interferes with bone acquisition in the important years before PBM.

We found that body weight and BMI had an influence on bone accretion as indicated both in previous cross-sectional TFF reports [136], reviews [113] and a comprehensive meta-analysis [114]. However, van Leeuwen and colleagues pointed out that the meta-analysis comprised studies that were mainly cross-sectional and only one longitudinal study exploring the long-term consequences of childhood obesity was included. The scope of our study was not

restricted to obesity, but rather how body weight influence bone in a young healthy adolescent population. Cross-sectionally, underweight appeared to be associated with low aBMD at baseline and this pattern persisted during two years of follow up. Particularly among boys, the differences compared to “normal weight” was apparent at all measured skeletal sites. Our findings suggest that the influence of baseline weight status on ΔaBMD were limited in girls compared to boys and that weight change might be strongest among boys with low BMI. This sex difference may be attributed to maturation, degree of longitudinal growth during follow-up, and that modifiable factors are more influential in a growing skeleton.

We observed indications of the hypothesis of the threshold effect of BMI on bone accretion in boys as previous reported [252], however based on a small sample size. Boys in the obese category had, on average, lower aBMD levels than overweight did at TFF1 and this relationship persisted during follow-up. It has been hypothesized that this could be attributed to the hormonal influence of fat on bone.

We explored change in exposure of body weight and BMI. Whether weight change in a young healthy population is enough to evoke an adaptive bone response has been debated [67], but previous research is not easy to come by. Studies of weight change are dominated by weight loss interventions related to obesity, anorexia nevrosa, menopause and use of medication. In the adult population, reduction of BW and BMI could lead to net loss of aBMD [253]. In adulthood, the link between body weight and bone mass is dynamic with increments of bone mass if the body weight increases and decrease if weight is lost. Net bone loss has not been reported in an adolescent population, and we found no indication of this happening in the TFF population. Mechanisms behind this age dependence may be explained by genetic driven skeletal growth and better muscle function. The accretion rate, however, did slow down and we observed a significant difference in ΔaBMD between BMI losers and BMI gainers.

Our results indicate that bone adapts to weight changes, but on average the influence during two years in late adolescence is limited. Exploring the effect of weight change on bone mass in obese female adolescents, Rourke and colleagues (41) found no bone loss, but concluded that reduction of BW induced a reduced bone growth rate over 12-month follow-up—results that are comparable to our findings. The effect of weight reduction on bone depends on whether it is voluntary or involuntary, the rate of change, age, sex, and initial weight (37). In

the current study, we had no information on the reason for BW changes, whether it was based on dieting, disease/illness, or natural fluctuations.

The issue of temporality can be discussed. As mentioned in the methodology discussion in section 5.1, baseline measures are a snapshot in a continuum and body weight is a part of the mechanical load and the skeleton continuously adapts throughout life. There is, however, a temporal lag of influence when association between change and some predictor is assessed. Changes in body weight would precede adaptations in bone, and without knowledge about when the weight change have occurred, the interpretations gets harder. Only two time points also limits the information on the actual relationship.

BMI is an extensively used tool to assess body weight adequacy, but the use of this parameter as a predictor of bone accretion is questionable. Its main advantage is simplicity and accessibility in a clinical setting, compared to other body composition measuring techniques (e.g. DXA). However, the mechanisms behind the relationship between weight status and bone are complex and multifactorial. BMI reflects both muscle and adiposity, which have different influence on bone. In a young healthy population it is reasonable to assume that excess BMI on average is attributed to excess fat mass, but it is not straightforward. The accuracy of BMI in this study population needs to be questioned because of longitudinal growth, particularly in boys. It is also worth recognizing that parts of the study population were elicited from sport schools and BMI does not account for over-average amounts of lean mass.

6.4 Association between use of tobacco and change in BMD/BMC

In this study all participants that responded on use of tobacco at both surveys were included, 349 girls and 281 boys. In girls, we compared 244 “never-users” with 105 “users” of snuff. In boys, 185 individuals were compared with 96, respectively. Use of snuff was associated with a lower rate of aBMD accretion during two years of follow-up in late adolescence among boys, but not among girls. In boys, the combined categories “daily” and “sometimes” users of snuff was associated with approximately 1 % lower ΔaBMD compared to “non-users”. Along with chronological age, use of snuff were one of the strongest crude determinants of bone accrual in the TFF cohort.

Smokeless tobacco has been linked to osteoporosis because of the detrimental effect of smoking on bone [254], however there is a wide range of types of smokeless oral tobacco products e.g. chewing tobacco, dry or moist types, with or without teabags etc. Some studies indicate that smokeless tobacco (chewing tobacco, non-combustible tobacco) is detrimental to bone in various populations, typically in India [255], Turkey [256] and in older multi-ethnic women [257]. It has been argued though, that Swedish snuff has a lower potential of harm than many other types of smokeless tobacco [258], and thus may not be comparable with studies from other populations and geographic areas. Apart from the cross-sectional study by Winther et al based on TFF1 data [136], we were unable to find other studies of the relationship between Swedish snuff and bone health in adolescence.

The study comprises adolescents between the age range 15 to 19 years of age, and this is an appropriate age group to explore the influence of use of snuff in terms of the temporal sequencing of exposure and outcome considering the mean onset among the participants were 14.3 years of age. We compared naturally occurring groups, thus there were no reason to expect equal baseline parameters. The baseline aBMD parameters did, however, not differ significantly between “non-users” and “users” of snuff groups.

A high degree of drift in the “sometimes” categories, both in regards to category stability and frequency, made sensitivity analysis with exclusion of this category the most sensible decision. Sensitivity analyses revealed that in girls, use of snuff appeared detrimental for femoral accretion when comparing “non-users” and “daily-users”. In boys, associations were strong in crude models, but were attenuated when controlling for relevant confounders, particularly changes in anthropometry, which may indicate that recreational drug use habits may be a marker for maturational aspects. One of the great challenges with the study of tobacco and bone is disentangling the influence of use from other lifestyle factors, because users of tobacco tend to possess other characteristics associated with low aBMD e.g. physical activity, alcohol etc. [12].

The findings of the limited associations between smoking and bone accretion may be due to the low prevalence of regular smoking. Only eight girls and eight boys reported daily smoking. Low prevalence of smokers in adolescence frequently limits statistical power [12].

This also made it difficult to investigate any dose-response relationship. In an extensive systematic review of PBM and its determinants, Weaver et al found evidence that supported smoking may have a deleterious influence on PBM [12]. However, there were some contradictory findings. The inconsistency in associations may be due to various categorization of smoking status employed or frequency- and duration-dependent effects of smoking on bone. We chose to exclude responses “one or less a week” and ended up with 21 girls and 31 boys in the “smokers” category for comparison with the “non-smokers”. The rationale behind this threshold could be debated, and was not based on any guidelines for what could be considered a “substantial” exposure that could influence the skeleton. The decision was mainly based on low prevalence of daily smokers. Category- and frequency fluctuations in the baseline “sometimes” categories also rises questions of the precision of the constructed “double-users”. The group of “double-users” may be considered even more ambiguous as it represents mainly “some-timers” for both smokers and users of snuff. However, the point estimates in all models were, with a few exceptions, negative for both use of snuff, smoking and double use, even though the statistical significance varied and depended on skeletal site and sex.

7 Conclusion

In summary, we have described changes in aBMD and BMC in Norwegian adolescents between 15 to 19 years of age and explored the influence of age, body weight, BMI, smoking and snuffing on PBM achievement. The main findings of this thesis were that:

- Girls between 17 and 19 years of age are approaching PBM at femoral sites.
- Boys are still accumulating bone mass between 17 and 19 years of age.
- There is a high degree of tracking of bone traits during 2 years in late adolescence and drift between quartiles is limited.
- Body weight and BMI are associated with bone accretion in late adolescence, but in a healthy young population, the modest magnitude of changes limits the implications for adolescents with an adequate body weight. However, low body BMI is associated with low aBMD and particularly among boys with low BMI, an increase in BMI could be beneficial for bone health.
- Use of snuff and double use are associated with lower rate of bone accretion in boys, but its relation to maturation requires further investigation.

Although the changes in aBMD and BMC associated with body weight and BMI are marginal and maybe not clinically relevant during two years of follow-up, there is a potential additive effect, which may be significant at a life-long perspective. The fact that we observed determinants that represent 1-2 % change during a mean time frame of 24 months, shows that lifestyle factors play an essential part of the PMB concept in late adolescence. Combined with other hereditary and modifiable determinants at this age, they may accumulate to an advantage, or a disadvantage, that represent a substantial effect later in life. An individual with low BMI that loses weight, smokes or uses snuff combined with other known determinants of low bone mass (e.g. physical activity/sedentary behavior) could be at great risk of less than optimal PBM, and eventually higher risk of osteoporosis and fragility fractures.

A better understanding of determinants of bone accrual and their relationships, increases the possibility of identifying individuals at risk of low PBM, with potential facilitation of early interventions and preventive measures.

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Paper I

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ORIGINAL ARTICLE

Changes and tracking of bone mineral density in late adolescence: the Tromsø Study, Fit Futures

Ole Andreas Nilsen[1] · Luai Awad Ahmed[1] · Anne Winther[2] · Tore Christoffersen[1] · Anne-Sofie Furberg[3] · Guri Grimnes[4,5] · Elaine Dennison[6,7] · Nina Emaus[1]

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Abstract
Summary Areal bone mineral density (aBMD) predicts future fracture risk. This study explores the development of aBMD and associated factors in Norwegian adolescents. Our results indicate a high degree of tracking of aBMD levels in adolescence. Anthropometric measures and lifestyle factors were associated with deviation from tracking.
Purpose Norway has one of the highest reported incidences of hip fractures. Maximization of peak bone mass may reduce future fracture risk. The main aims of this study were to describe changes in bone mineral levels over 2 years in Norwegian adolescents aged 15–17 years at baseline, to examine the degree of tracking of aBMD during this period, and to identify baseline predictors associated with positive deviation from tracking.
Methods In 2010–2011, all first year upper secondary school students in Tromsø were invited to the Fit Futures study and 1038 adolescents (93%) attended. We measured femoral neck (FN), total hip (TH), and total body (TB) aBMD as g/cm^2 by DXA. Two years later, in 2012–2013, we invited all participants to a follow-up survey, providing 688 repeated measures of aBMD.
Results aBMD increased significantly ($p < 0.05$) at all skeletal sites in both sexes. Mean annual percentage increase for FN, TH, and TB was 0.3, 0.5, and 0.8 in girls and 1.5, 1.0, and 2.0 in boys, respectively ($p < 0.05$). There was a high degree of tracking of aBMD levels over 2 years. In girls, several lifestyle factors predicted a positive deviation from tracking, whereas anthropometric measures appeared influential in boys. Baseline z-score was associated with lower odds of upwards drift in both sexes.
Conclusions Our results support previous findings on aBMD development in adolescence and indicate strong tracking over 2 years of follow-up. Baseline anthropometry and lifestyle factors appeared to alter tracking, but not consistently across sex and skeletal sites.

Keywords Bone mass · Bone development · Tracking · Adolescence · Areal bone mineral density · DXA

✉ Ole Andreas Nilsen
ole-andreas.nilsen@uit.no

[1] Department of Health and Care Sciences, UiT The Arctic University of Norway, 9019 Tromsø, Norway

[2] Division of Neurosciences, Orthopedics and Rehabilitation Services, University Hospital of North Norway, Tromsø, Norway

[3] Department of Community Medicine, UiT The Arctic University of Norway, 9019 Tromsø, Norway

[4] Division of Internal Medicine, University Hospital of North Norway, 9019 Tromsø, Norway

[5] Endocrine Research Group, Department of Clinical Medicine, The Arctic University of Norway, 9019 Tromsø, Norway

[6] MRC Lifecourse Epidemiology Unit, Southampton, UK

[7] Victoria University, Wellington, New Zealand

Introduction

Norway has one of the highest reported incidences of hip fractures [1]. Areal bone mineral density (aBMD) is strongly associated with fracture risk. aBMD levels in the elderly are a result of peak bone mass (PBM) achieved during growth and subsequent bone loss [2]. Adolescence is characterized by massive skeletal changes due to rapid modeling and remodeling [3]. About 40% of bone mass are accumulated around the 4 years of peak height velocity (PHV) during puberty and about 90% by the age of 18 [4, 5]. These rapid changes generate both opportunities and vulnerabilities related to future bone health. Previous studies indicate that one standard deviation increase in bone mass at the end of skeletal maturation decrease future fracture risk by as much as

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50% [4]. This makes maximization of the genetic potential for bone mass acquisition a strategy for prevention of osteoporosis and fragility fractures later in life. The clinical importance of this concept depends on the degree of tracking or stability of bone mineral status from younger years into adulthood [6]. Early preventive measures can be employed if there is a high correlation between bone mass levels in the younger years and later in life. Studies report that high aBMD in athletes or low aBMD due to deficits may persist into adulthood [7, 8]. Previous population-based longitudinal studies demonstrate strong tracking of aBMD from childhood to skeletal maturity [9–13]. The degree of tracking from adolescence into adulthood is, however, unclear [14–16]. Potential variation in tracking into adulthood and inconsistent evidence [10–12] calls for attention to predictors of deviation from tracking in late adolescence. The objectives of this population based longitudinal study were (1) to describe the changes in bone traits over 2 years in Norwegian adolescents aged 15–19 years, (2) to explore tracking of aBMD status over 2 years, and (3) to identify baseline anthropometric measures and lifestyle factors associated with deviation from tracking. It is our hypothesis that participants mainly remain in their original aBMD quartile between the ages of 15 and 19 years of age and that baseline predictors of positive deviation from tracking can be detected.

Methods

Subjects

The Tromsø Study is an ongoing population-based epidemiological study with seven repeated surveys conducted among the adult population since 1974 [17]. As part of the Tromsø Study, Fit Futures invited all first year upper secondary school students in Tromsø and the neighboring municipalities to a comprehensive health survey in 2010–2011 (TFF1, baseline). The invited cohort comprised 1117 adolescents and 1038 (508 girls and 530 boys) attended the survey (attendance rate 93%). Among those, 95% of the participants were in the range between 15 and 18 years of age. Two years later, in 2012–2013, all third year upper secondary school students in the same schools and all TFF1 participants not attending school at that time were invited to a follow-up survey, Fit Futures 2 (TFF2). In total, 820 adolescents attended, providing 688 repeated measures of aBMD (66% of the TFF1 cohort) (Fig. 1). The Clinical Research Unit at the University Hospital of North Norway conducted both surveys during school days. The Regional Committee of Medical Research Ethics approved the study (Ref. 2013/1459/REK nord). The study protocol for TFF1 was approved by The Norwegian Data Inspectorate 27.07.2010 (Ref. 07/00886-7/CGN) and the Regional Committee for Medical Research Ethics (REK-Nord) 16.09.2010 (Ref. 2009/1282-23). The study protocol for TFF2 was approved as an extension of the prior approval by the Data Inspectorate 31.10.2012 (Ref. 07/00886-15/EOL). All participants gave written informed consent. Participants below 16 years of age had to bring written consent from their superiors to attend the survey.

Measurements

We measured total body (TB), total hip (TH), and femoral neck (FN), bone mineral content (BMC; g), bone area (BA; cm^2), and aBMD (g/cm^2) by DXA (GE Lunar prodigy) and performed analyses by Encore pediatric software [18]. The densitometer coefficient of variation (CV = [SD/mean] × 100) has been estimated to 1.14% at the total hip measured in vivo [19]. We used the same densitometer in both surveys, and no densitometer drift was detected between the surveys. Trained technicians performed the measurements, and the quality assessment was done according to the same protocol in both surveys. We used measurements of left hip at both femoral sites. In 15 cases, left hip data was missing and the right hip was used. Measurements from the same hip were used in both TFF1 and TFF2. Height and weight were measured to the nearest 0.1 cm and 0.1 kg on the same electronic scale in both surveys (Dong Sahn Jenix, Korea), with participants wearing no shoes and light clothing. We assessed use of medication, acute and chronic diseases, hormonal contraceptive use, and the possibility of pregnancy by clinical interviews, and pregnant participants were excluded from DXA scanning. Participant's answers on diseases and use of medication known to affect bone were operationalized into dichotomous variables. Hormonal contraceptive use were categorized into no use, combined estrogen and progestogen-based contraceptive (CHC) use, and progestogen-only contraceptive use. We collected sexual maturation information by self-administered questionnaires. In girls, pubertal status was determined through the following questions: "If you have started menstruating, how old were you when you had your first menstruation." Answers were categorized into "early" (<12.5 years at menarche), "intermediate" (12.5–13.9 years), or "late" (>14 years) sexual maturation. Boys were examined according to Pubertal Developmental Scale (PDS). The boys self-rated secondary sexual characteristics as growth spurt, pubic hair growth, changes in voice, and facial hair growth on a scale from 1 (have not begun) to 4 (completed). We summarized the score and divided by 4. We categorized a score <2 as "have not begun," 2–2.9 as "barely started," 3–3.9 as "underway," and a score of 4 as "completed" [20]. The participants were asked to grade leisure time physical activity (PA) in an average week during the last year according to a four-level scale, which are sedentary activities only; moderate activity like walking, cycling, or exercise at least 4 h per week; participation in recreational sports at least 4 h per week; or participation in hard training/sports competitions several times a week. This question was developed by Saltin and Grimby

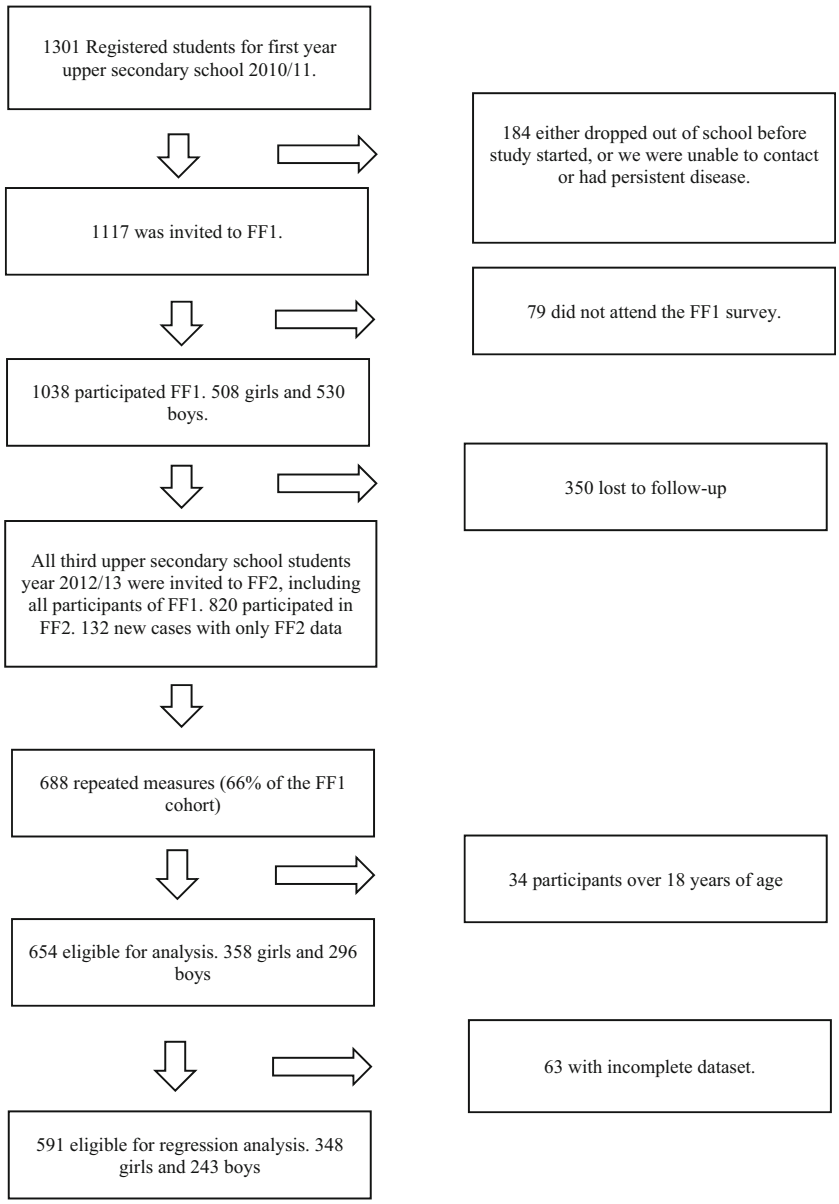

Fig. 1 Flowchart of participation in Fit Futures 1 (TFF1) 2010–2011 and Fit Futures 2 (TFF2) 2012–2013

[21] and has previously been validated in the Tromsø Study [22]. Questions on smoking and snuffing had the following three alternatives: never, sometimes, or daily, while frequency of alcohol consumption had the following five alternatives: "never," "once per month or less," "two to four times per month," "two to three times per week," and "four or more times per week." We dichotomized answers on smoking, snuffing, and alcohol into yes and no.

Statistical analyses

All analyses were performed sex stratified. We calculated means and standard deviations for continuous variables and percentage for categorical variables to describe the study population characteristics. Differences in anthropometric and DXA measures between FF1 and TFF2 were tested using paired sample *t* test, while dichotomous lifestyle factors were tested with McNemar's test. We explored differences between participants and non-responders in TFF2 using Student's *t* test and chi-squared testing. Average absolute change and percentage change for BMC and aBMD for each skeletal site were calculated by the difference between the measurements ($T_2 - T_1$). We used exact measurement dates to compute annual change to account for differences in time between measurements. We stratified participants by age and used one-way ANOVA and multiple comparisons with Bonferroni post hoc test to examine differences in mean aBMD change between groups. We calculated individual age and sex-specific height, weight, FN, TH, and TB aBMD and BMC z-scores (standard deviations away from the sample specific mean) and examined correlations between baseline and follow-up using Pearson's correlation coefficient. Because height and weight are known determinants of aBMD and the adjustment for height in

Table 1 Characteristics at baseline survey Fit Futures 1 (TFF1) and follow-up survey Fit Futures 2 (TFF2) 2 years later: continuous variables presented as mean (standard deviation) and categorical variables in percentage

	Girls					Boys				
	TFF1		TFF2			TFF1		TFF2		
	n		*n*		*p*	*n*		*n*		*p*
Age	358	16.61 (0.387)	358	18.60 (0.40)		296	16.60 (0.367)	296	18.65 (0.35)	
Age groups at baseline										
15	9	2.5%				19	6.4%			
16	296	82.7%				238	80.4%			
17	53	14.8%				39	13.2%			
Height (cm)	358	165.07 (6.47)	358	165.77 (6.56)	<0.001	296	177.25 (6.52)	296	179.08 (6.49)	<0.001
Weight (kg)	358	60.42 (10.61)	358	63.11 (11.91)	<0.001	296	69.81 (13.68)	296	75.21 (14.64)	<0.001
Sexual maturation[a]										
Early/completed	110	31.3%				22	9.1%			
Intermediate/underway	168	47.9%				177	72.8%			
Late/barely started	73	20.8%				44	18.1%			
Ethnicity										
White	350	97.8%				291	98.3%			
Others	8	2.2%				5	1.7%			
Physical activity										
Sedentary	43	12.0%	47	13.3%		77	26.3%	81	28.4%	
Moderate	141	39.5%	144	40.8%		75	25.6%	60	21.1%	
Sports	110	30.8%	110	31.2%		71	24.2%	77	27.0%	
Competition	63	17.6%	52	14.7%		70	23.9%	67	23.5%	
Smoking (yes)	68	19.0%	102	28.5%	<0.001	62	20.9%	114	38.5%	<0.001
Snuff use (yes)	108	30.2%	152	42.5%	<0.001	108	36.5%	142	48.0%	<0.001
Alcohol consumption (yes)	262	73.2%	336	93.9%	<0.001	195	65.9%	272	91.9%	<0.001
Diseases known to affect bone[b] (yes)	4	1.1%				5	1.7%			
Medication known to affect bone[c] (yes)	8	2.2%				6	2.0%			
Hormonal contraceptive use (yes)	118	33.0%								
Estrogen and progestogens	105	29.3%								
Progestogens only	13	3.6%								

[a] Sexual maturation in girls: menarche age. Categories are early (<12.5), intermediate (12.5–13.9), and late (>14). Sexual maturation in boys: Puberty Developmental Scale. Categories are have not begun (<2), barely started (2–2.9), underway (3–3.9), and completed (4)

[b] Diseases known to affect bone (ICD10): E03 hypothyroidism, E10 diabetes type 1, F50.9 eating disorders, K90.0 celiac disease, and M13 arthritis

[c] Medication known to affect bone (ATC): D07A plain corticosteroids, H03A thyroid preparations, N03A antiepileptic, R01AD corticosteroids, R03BA glucocorticoids (inhalants), and H02A corticosteroids for systemic use

the two-dimensional DXA scans is incomplete, partial correlation was used to adjust for TFF1 height and weight as well as change in height and weight. We stratified participants into quartiles of aBMD and BMC z-scores and examined the proportions of participants that remained within quartiles, drifted upwards, or drifted downwards between TFF1 and TFF2. Furthermore, an aBMD z-score change variable were computed ($Z_2 - Z_1$). To test whether baseline age, anthropometric traits (height, weight), and lifestyle factors (PA, alcohol consumption, smoke use, and snuff use) were associated with positive deviation from tracking (z-score change >0), we used logistic regression. The reference categories were no change or downwards drift (z-score change ≤0). Odds ratios (ORs) with 95% confidence intervals (CIs) for upwards drift during follow-up were calculated. We simultaneously adjusted for age, anthropometric measures, lifestyle variables, sexual maturation, and time between measurements. The influence of other relevant confounders like baseline aBMD z-score, ethnicity, chronic disease, and medication known to affect bone health bone and hormonal contraceptive use (girls) were explored, and purposeful selection was used to select final model [23]. We evaluated relevant two-way interactions. We fitted models for FN, TH, and TB separately and ran logistic regression diagnostics, and assumptions were met. Significance level was set to $p = 0.05$ in all analysis, and all procedures were performed in SPSS version 23.

Results

Descriptives

We included 654 adolescents, 358 girls and 296 boys aged 15 to 17 at baseline in the present analysis (Table 1). The majority were 16 years of age ($n = 534$), while a small group of 28 participant were 15 years at baseline. Mean follow-up time was 1.94 years (SD 0.20). Thirty-two percent of TFF1 participants were lost to

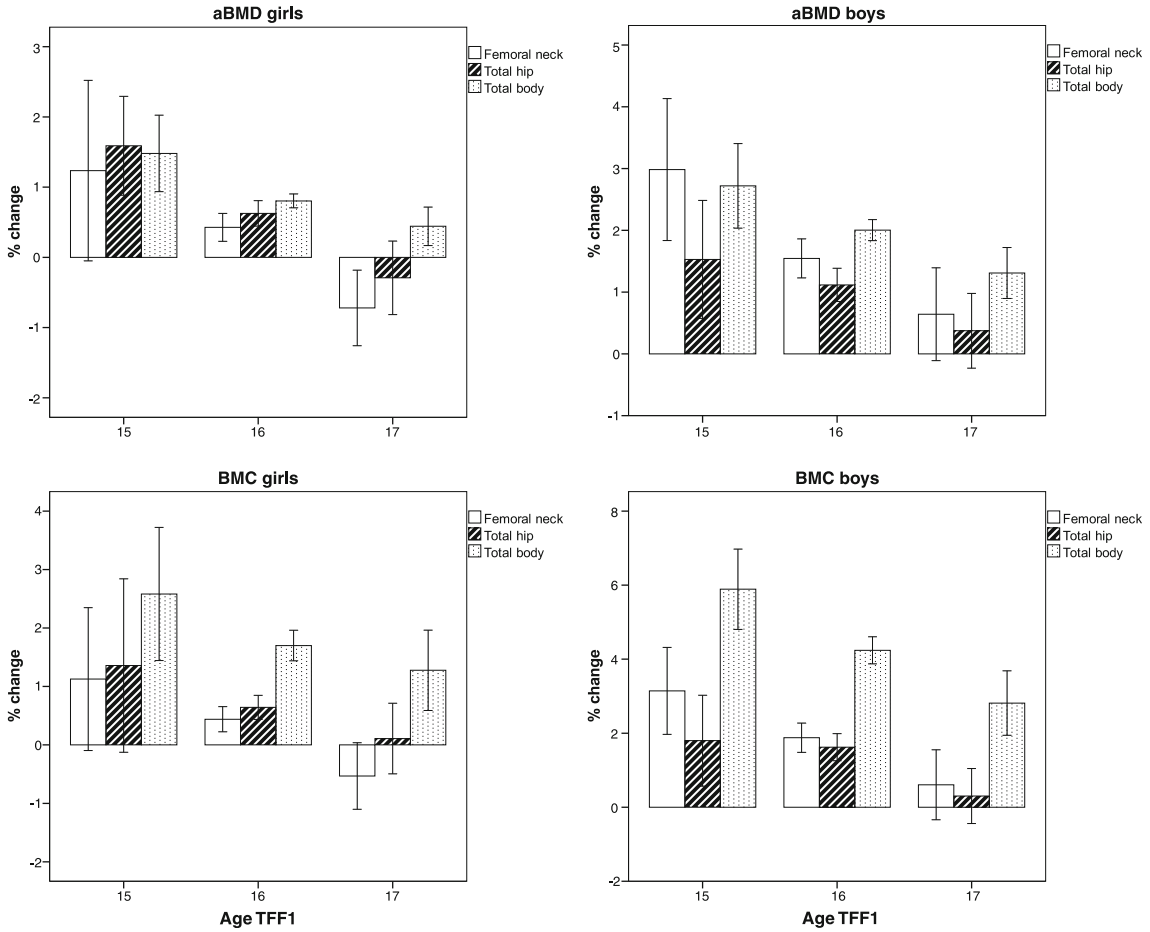

Fig. 2 Mean annual percent change in femoral neck total hip and total body aBMD and BMC for girls and boys stratified by age at Fit Futures 1 (TFF1) with 95% confidence intervals

follow-up. Dropout analysis showed statistically significant higher proportion of boys, smokers, snuff users, and consumers of alcohol (girls only) among non-responders compared to those who participated in both surveys.

Changes in bone traits and anthropometry

In the overall study, population aBMD increased significantly ($p < 0.05$) at all sites in both sexes. Mean annual percentage increase for FN, TH, and TB aBMD (g/cm^2) was 0.3, 0.5, and 0.8 in girls and 1.5, 1.1, and 2.0 in boys, respectively ($p < 0.05$). A similar pattern was present for BMC. When stratified into age at baseline, mean annual percent change in aBMD at all skeletal sites decreased successively by increasing age in both sexes (Fig. 2). The differences in annual aBMD changes between age groups were statistically significant ($p < 0.05$) at most skeletal sites and ages; the exceptions were changes in TH aBMD between all age groups and FN aBMD between age 16 and 17 years in boys, as well as changes in FN and TH aBMD between 15- and 16-year-old girls. Girls 17 years of age at TFF1 had a mean annual percentage FN aBMD loss of −0.61 (95% CI −0.15, −1.07) and −0.14 (−0.54, 0.27) at the total hip. Average annual percentage BA change for FN, TH, and TB were 0.01, 0.09, and 2.30 and 0.23, 0.39, and 2.10 for girls and boys, respectively. The average annual height and weight changes during the follow-up period were 0.36 cm (95% CI 0.32–0.41) and 1.37 kg (1.11–1.63) for girls and 0.93 cm (0.83–1.03) and 2.70 kg (2.35–3.04) for boys, respectively.

Tracking from baseline to follow-up

Correlations between TFF1 and TFF2 z-scores were high in both sexes at aBMD FN, TH, and TB, Pearson's $r = 0.960$, 0.966, and 0.967 for girls and 0.937, 0.955, and 0.946 for boys, respectively. Calculations of coefficients for BMC, height, and weight showed similar strong correlations. Adjusting for TFF1 height and weight or changes in height and weight using partial correlation did not change the aBMD results (not shown). Age-stratified coefficients showed weaker correlation at all sites for 15-year-old boys, FN 0.884, TH 0.871, and TB 0.853 ($N = 19$). All correlation coefficients were statistically significant ($p < 0.0001$). Overall, 78.2% of the girls kept their FN aBMD quartile position between

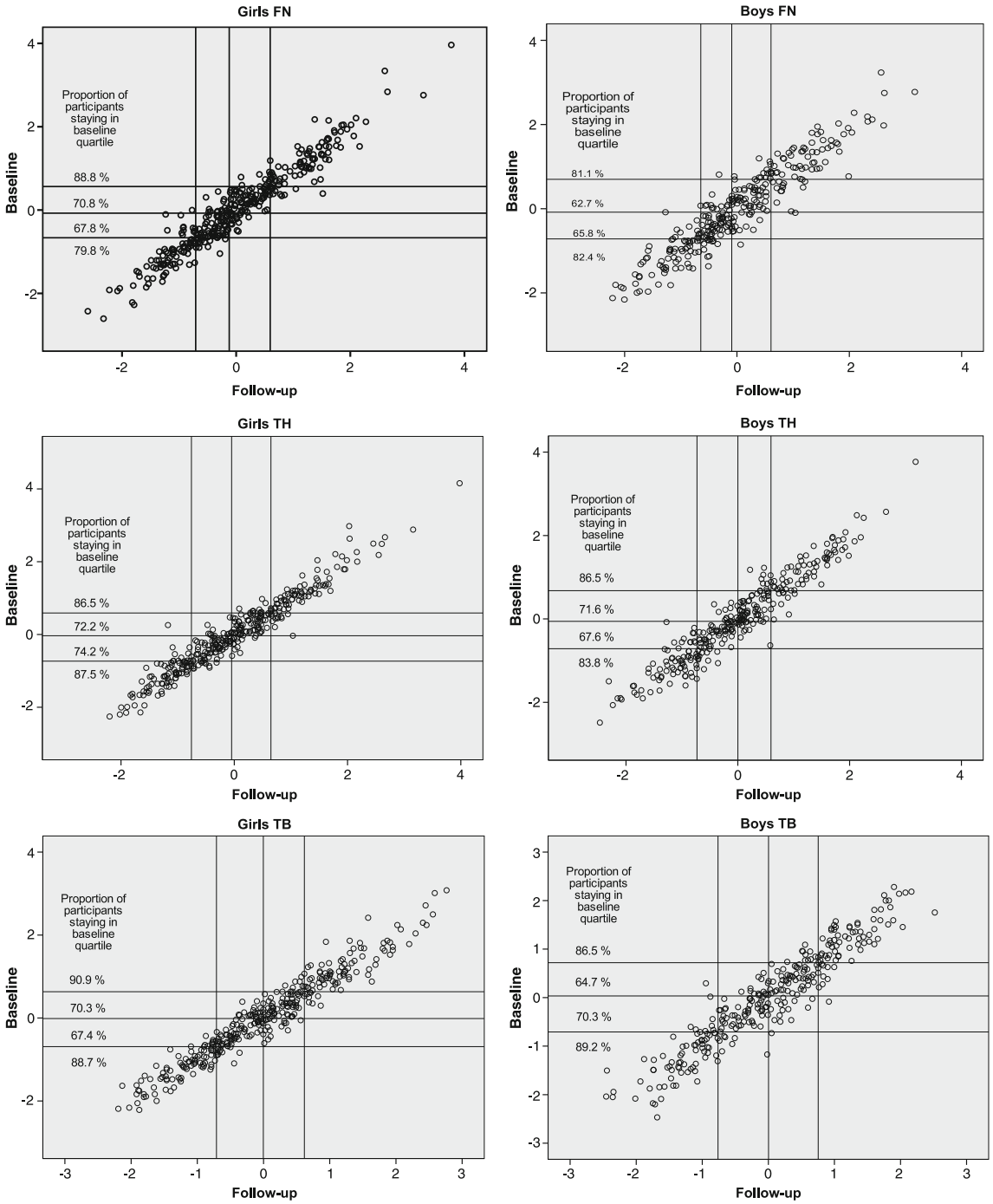

Fig. 3 Scatterplot of aBMD z-score for femoral neck (FN), total hip (TH), and total body (TB) at baseline vs z-score at follow-up with proportions of participants remaining in baseline quartile. *Lines* represent the cutoff for percentiles 25, 50, and 75%. Measurements outside diagonal quartiles have changed quartile between baseline and follow-up. Participants were 15–17 years of age at baseline. Boys $n = 296$. Girls $n = 358$

measurements, correspondingly 73% of the boys. The same stability within quartiles was found at TH and TB, 79.6 and 77.4% for girls and 79.2 and 77.7% for boys, respectively. Figure 3 illustrates z-score drift between baseline, and follow-up and shows proportions of participants remaining in each specific quartile.

Predictors of positive deviation from tracking

Baseline FN, TH, and TB aBMD z-scores had a statistically significant association with lower odds of positive deviation from tracking for both girls and boys (Table 2). Later sexual maturation tended to be associated with higher odds of

Table 2 Mean and (standard deviation) of bone traits and time between measurements: areal bone mineral density (aBMD), bone mineral content (BMC), and bone area (BA) for femoral neck (FN), total hip (TH), and total body (TB) at baseline survey Fit Futures 1 (TFF1) and follow-up survey Fit Futures 2 (TFF2) 2 years later

	Girls					Boys				
	TFF1		TFF2			TFF1		TFF2		
	n		*n*		*p*	*n*		*n*		*p*
aBMD FN (g/cm^2)	358	1.07 (0.13)	357	1.08 (0.13)	0.008	296	1.11 (0.15)	296	1.14 (0.15)	<0.001
aBMD TH (g/cm^2)	357	1.06 (0.13)	357	1.07 (0.13)	<0.001	296	1.12 (0.15)	296	1.14 (0.16)	<0.001
aBMD TB (g/cm^2)	357	1.14 (0.08)	358	1.16 (0.07)	<0.001	296	1.18 (0.10)	296	1.23 (0.09)	<0.001
BMC FN (g)	358	4.92 (0.71)	357	4.94 (0.72)	<0.001	296	5.99 (0.99)	296	6.19 (0.99)	<0.001
BMC TH (g)	357	32.03 (4.84)	357	32.42 (4.95)	<0.001	296	40.17 (6.64)	296	41.26 (6.86)	<0.001
BMC TB (g)	357	2524.06 (388.27)	358	2600.95 (381.68)	<0.001	296	2963.78 (469.83)	296	3200.96 (476.10)	<0.001
BA FN (cm^2)	358	4.60 (0.34)	357	4.60 (0.34)	0.866	296	5.38 (0.39)	296	5.41 (0.37)	0.003
BA TH (cm^2)	357	30.15 (2.32)	357	30.22 (2.38)	0.068	296	35.73 (2.47)	296	35.99 (2.51)	<0.001
BA TB (cm^2)	357	2207.37 (233.59)	358	2241.68 (224.95)	<0.001	296	2496.46 (240.06)	296	2598.28 (237.87)	<0.001
Time between measurements (years)	358	1.94 (0.20)				296	2.01 (0.23)			

positive drift at several skeletal sites, with a statistically significant association for TB in girls. For boys, baseline body weight was associated with higher odds of positive deviation at TH (p = 0.018), and a statistically significant interaction between age and weight was detected at FN; when stratified into younger (<16.66 years) and older (≥16.67 years) boys, the association between baseline weight and higher odds of positive deviation in FN aBMD was limited to the younger boys (p = 0.039). There were no statistically significant associations between lifestyle factors and higher aBMD z-scores in boys; smoking only tended to be associated with decreased odds for higher TH aBMD z-score at follow-up (p = 0.062). In girls, snuff and alcohol use were associated with significantly lower odds of higher TH and TB aBMD z-scores, respectively. Also, CHC use was associated with reduced odds of upwards drift during follow-up at FN (p = 0.048). Baseline recreational PA level was positively associated with significantly higher TB aBMD z-score at follow-up in girls; participation in recreational sports at least 4 h per week and participation in hard training/sports competitions several times a week were associated with a fourfold and threefold increase in the odds of higher TB aBMD, respectively. Data also indicated a more moderate effect of PA on FN aBMD in girls (p = 0.080; Table 3).

Discussion

This study presents results from a large population-based cohort of adolescents entering young adulthood. Our results indicate that Norwegian adolescents still accumulate bone mass and increase aBMD between 16 and 18 years of age, although bone acquisition decreases significantly with age at all skeletal sites during these 2 years of follow-up. The results also suggest that girls may be reaching an aBMD plateau at femoral sites between 17 and 19 years of age, even with an indicated reduction of aBMD at femoral neck around the age of 19 compared to 2 years earlier. Consistent with our hypothesis, we report that a stable position within quartiles based on aBMD z-scores is kept over 2 years in late adolescence. Baseline z-scores were consistently associated with lower odds of positive deviation from tracking across all skeletal sites for both sexes. In boys, anthropometric baseline measures appeared to be associated with upwards drift. In girls, several lifestyle factors had statistically significant associations. Particularly, PA tended to be beneficial for TB aBMD.

The decrease in FN aBMD for girls between 17 and 19 years of age is unexpected. However, Berger et al. reported similar findings with an average decrease of aBMD in girls around 20 years of age until stabilization and consolidation [24]. As no specific characteristic in these girls could account for this development like late menarche or intensive physical activity, the relationship between BMC and BA and precision of measurement could explain these findings. According to Sundberg et al. [25], pubertal bone growth is due to increased bone size rather than increased density. aBMD will increase only if BMC increases proportionally more than BA [4]. Elaborative analysis showed that mean FN BA in girls aged 17 years at baseline increased while mean BMC dropped slightly resulting in lower mean aBMD. The decreasing trend of bone acquisition with age is similar at all three sites, and changes in femoral sites seem to drop in advance of total body aBMD. This is consistent with other longitudinal studies [26,

Table 3 Baseline anthropometric measures and lifestyle factors associated with positive deviation from tracking (z-score change >0) over 2 years in late adolescence

	Girls						Boys					
	FN (n = 183 vs 167)		TH (n = 182 vs 167)		TB (n = 170 vs 180)		FN (n = 117 vs 123)		TH (n = 127 vs 114)		TB (n = 112 vs 129)	
	OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p
Age (year)	0.64 (0.35, 1.16)	0.142	*0.43 (0.24, 0.79)*	*0.007*	0.71 (0.39, 1.29)	0.265	[a]		0.56 (0.23, 1.37)	0.205	0.56 (0.23, 1.38)	0.211
Height (cm)	1.00 (0.96, 1.04)	0.855	1.01 (0.97, 1.04)	0.805	1.02 (0.98, 1.06)	0.390	1.00 (0.95, 1.05)	0.988	1.00 (0.95, 1.05)	0.988	*1.06 (1.01, 1.11)*	*0.023*
Weight (10 kg[b])	1.00 (0.98, 1.03)	0.711	1.00 (0.82, 1.32)	0.744	1.23 (0.93, 1.61)	0.143	[a]		*1.36 (1.05, 1.76)*	*0.018*	1.21 (0.94, 1.57)	0.141
Z-score at baseline	*0.74 (0.58, 0.96)*	*0.022*	*0.74 (0.57, 0.97)*	*0.026*	*0.66 (0.49, 0.88)*	*0.005*	*0.67 (0.47, 0.94)*	*0.021*	*0.64 (0.45, 0.90)*	*0.011*	*0.50 (0.35, 0.72)*	*0.000*
Sexual maturation	Reference: menarche age <12.5 years		Reference: pubertal development completed									
Intermediate/underway	1.41 (0.84, 2.37)	0.198	1.24 (0.73, 2.11)	0.420	1.28 (0.75, 2.18)	0.376	2.55 (0.84, 7.74)	0.098	1.55 (0.56, 4.23)	0.399	1.09 (0.52, 5.87)	0.871
Late/just started	1.68 (0.88, 3.20)	0.114	1.68 (0.87, 3.21)	0.120	*2.05 (1.05, 3.98)*	*0.035*	2.44 (0.67, 8.61)	0.167	1.50 (0.46, 4.90)	0.502	1.74 (0.52, 5.87)	0.371
Hormonal contraceptive use	Reference: no contraceptive use											
Estrogen and progestogen	*0.60 (0.36, 1.00)*	*0.048*										
Progestogen only	0.79 (0.24, 2.55)	0.687										
Physical activity	Reference: sedentary											
Moderate	1.94 (0.93, 4.06)	0.080	1.52 (0.72, 3.21)	0.278	1.78 (0.82, 3.86)	0.147	0.62 (0.27, 1.40)	0.276	0.61 (0.26, 1.39)	0.237	0.53 (0.23, 1.20)	0.128
Sports	1.79 (0.82, 3.90)	0.143	1.64 (0.75, 3.61)	0.217	*4.07 (1.78, 9.30)*	*0.001*	0.56 (0.24, 1.30)	0.138	0.80 (0.34, 1.85)	0.594	0.71 (0.31, 1.62)	0.414
Competition	1.95 (0.80, 4.72)	0.140	1.24 (0.51, 3.01)	0.640	*3.28 (1.31, 8.20)*	*0.011*	0.83 (0.35, 2.00)	0.680	0.70 (0.29, 1.73)	0.443	1.45 (0.62, 3.40)	0.390
Snuff use [c]	0.93 (0.52, 1.66)	0.802	*0.50 (0.28, 0.89)*	*0.019*	1.09 (0.61, 1.94)	0.769	0.77 (0.35, 1.68)	0.513	0.71 (0.32, 1.54)	0.384	0.61, (0.27, 1.35)	0.221
Smoking [c]	0.80 (0.42, 1.54)	0.510	1.03 (0.54, 1.99)	0.919	1.17 (0.61, 2.34)	0.645	0.53 (0.22, 1.30)	0.166	0.43 (0.18, 1.04)	0.062	0.47 (0.19, 1.15)	0.097
Alcohol consumption [c]	1.03 (0.60, 1.78)	0.913	1.24 (0.71, 2.15)	0.450	*0.45 (0.26, 0.80)*	*0.006*	0.87 (0.45, 1.67)	0.674	0.84 (0.43, 1.64)	0.611	1.55 (0.80, 2.99)	0.193

Odds ratios (OR) for femoral neck (FN), total hip (TH), and total body (TB) with confidence intervals (CIs). Reference group were no change or negative deviation from tracking (z-score change ≤0). All the variables are mutually adjusted for other variables in the model including time between measurements. $P < 0.05$ in italics

[a] Significant interaction between age and weight $p = 0.022$. When stratified by younger/older age <16.66 years, ORs for weight were 1.49 (95% CI 1.02, 2.18), $p = 0.039$, $n = 52$ vs 68, and age ≥16.67 years, OR for weight 1.03 (0.67, 1.58), $p = 0.909$, $n = 71$ vs 41

[b] Associations with 10 kg change in body weight

[c] Yes/no

27]. Bachrach et al. found that, for girls, gains in aBMD leveled off in total hip, spine, and whole body already at the age of 14.1, 15.7, and 16.4, respectively. Boys tended to reach plateau at the age of 15.7 in total hip and 17.7 in spine and whole body [28]. Differences in statistical analysis used to localize the age of plateau may explain the slightly earlier age indication compared to our findings. The 2-year developmental difference between boys and girls was present in our cohort as well. Hormonal status influences bone development and PBM depends on biological rather than chronological age [29].

Our tracking results are comparable with other studies [10, 12, 13]. In contrast, Buttazzoni et al. [16] concluded with low sensitivity for childhood bone mass scans to predict PBM. Their study included 65 boys and 56 girls with a time frame of 11 years. With the extensive follow-up period and a mean baseline age of 8 years, this study is not directly comparable to ours. Follow-up during PHV is expected to show reduced correlation, and Kalkwarf et al. reported lower correlations in younger children than in older [10]. In our cohort, aBMD tracking for boys became successively stronger as annual height change reduced gradually between 15 and 17 years of age at baseline, indicating this link between statural growth and aBMD tracking (data not shown). The tendency of stronger degree of tracking with cessation of growth strengthens the notion that measures in our study potentially can predict adult bone mineral status. The results for participants in the lowest quartile are of clinical importance and highlight the great challenge of changing the bone mineral-level trajectory of this group. Even though this study has a narrow time span, the fact that a large proportion of adolescents with low bone mass levels remains low supports the hypothesis that subjects susceptible to relatively early osteoporosis risk may be detectable early in life.

The importance of PBM makes it interesting to explore modifiable factors with the potential of altering the bone mass trajectory. Our study suggested that baseline body weight may influence aBMD at femoral sites in boys, but not in girls. Age being an effect modifier of weight for boys at FN is biologically reasonable because bone adaptation to mechanical loading is greater in a growing skeleton and FN is highly exposed to weight [30]. No associations between lifestyle factors and positive drift were detected for boys. For girls, associations were incoherent both in terms of direction, statistical significance, and skeletal sites. PA seemed beneficial for TB aBMD, but we found no clear dose-response effect. This may indicate that participants reporting to be in the hard training and competition category at baseline were already at the tail of the z-score distribution as reported by Winther et al. [31]. Sustained activity level during follow-up and preservation of high z-score could lead to classification into the reference group no change or downwards drift for these participants. Previous studies report tobacco use to have a duration and dose-dependent negative effect on aBMD, while the impact of alcohol is more unclear [32–36]. Snuff use and smoking mainly prevented subjects from positive deviation in our study, although not statistically significant at all skeletal sites. However, changes in exposure variables during follow-up make the interpretations of associations challenging. Proportions of smokers, snuff users, and participants consuming alcohol all increased during follow-up (Table 1). The relationship between hormonal contraceptive use and aBMD development remains controversial. Our results indicated CHC use to be disadvantageous for the FN and supports evidence suggesting that CHC use is likely to impair acquisition of optimal PBM [37]. Recent reviews emphasize the need for randomized controlled trials to confirm these effects [38]. Progestogen-only contraceptives have also been associated with reduced aBMD when used before the achievement of PBM [39]. This association was not confirmed in our cohort, but participants reporting to use progestogen-only contraceptives were few. The underlying mechanisms behind the effects of contraceptives are complex and data on length of use and dosage are lacking. Winter et al. reported that late sexual maturation was associated with low aBMD levels in TFF1 [31]. The fact that proportions of sexual maturation categories in our study are comparable with other Norwegian youth cohorts [40] and that the association between late sexual maturation and increased odds for positive deviation in this longitudinal study is consistent suggest that this adverse effect levels out to some extent. As reported by previous studies [10, 12], baseline aBMD z-score appears to be highly predictive of future z-score. The consistent association between high baseline z-score and reduced odds of positive deviation could be due to the phenomenon regression towards the mean. Extreme measures at the tails of the distribution will when repeated tend to be less extreme and closer to average because of variation within the individual or measurement error [41].

The longitudinal design and the large representative sample are among the strengths of the study. The sample has well-described characteristics, is homogenous in age and ethnicity, and included both sexes and participants from both rural and urban regions. We used the same densitometer through both surveys with continuous validations. A well-established research unit ensured high quality of data acquisition. There are, however, limitations to be discussed. Firstly, DXA and aBMD measurements have their limitations. Interpretation of DXA measures of growing skeletons could be problematic because it is a two-dimensional measure and size dependent [42]. aBMD is furthermore only a surrogate measure of bone strength, and the broad concept of PBM captures other parameters like architecture, geometry, and distribution of trabecular and cortical bone [6]. Secondly, non-participation and loss to follow-up could be a problem if only the healthy part of the population chooses to participate. Fourteen percent of the eligible population were not invited because we were unable to get in contact with them due to chronic illness or dropout from

school. School dropouts tend be associated with an unhealthy lifestyle [35]. The detected differences in characteristics between non-responders and participants attending both surveys may cause bias. A higher proportion of snuff user among non-responders would make the statistically significant association between snuff use and lower odds for positive drift for girls an underestimation. Thirdly, we acknowledge that the follow-up time of 2 years may be a limitation. Changes over such a short time period are at risk of being obscured by variability in DXA measurements. On the other hand, the recommended minimum interval between DXA scans is 6–12 months [42], and our findings are in accordance with previous reports.

In conclusion, this study corroborates the findings of previous research exploring the dynamics of bone mineral levels in adolescence. We report a high degree of tracking of aBMD levels over 2 years in late adolescence. Because of the short time span between measurements, a longer follow-up is necessary for definite conclusions on tracking. Baseline aBMD z-score was the only consistent predictor of deviation from tracking in both girls and boys. For boys, baseline body weight tended to be associated with upwards drift in aBMD z-score at femoral sites. For girls, lifestyle factors such as PA, snuff use, and consumption of alcohol appeared important, but not persistently across skeletal sites. Further studies are needed in order to investigate the possible effect of changes in anthropometrics and lifestyle factors on development of aBMD in adolescence. Additional follow-up surveys of the Fit Futures cohort are required to explore further longitudinal effects.

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Compliance with ethical standards

Conflicts of interest None.

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Paper II

Nilsen OA, Ahmed LA, Winther A, Christoffersen T, Thrane G, Evensen E, Furberg AS, Grimnes G, Dennison E, Emaus N. Body weight and body mass index influence bone mineral density in late adolescence in a two-year follow-up study. The Tromsø Study, Fit Futures. *JBMR Plus. 2019 Aug 21;3(9):e10195.*

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Body Weight and Body Mass Index Influence Bone Mineral Density in Late Adolescence in a Two-Year Follow-Up Study. The Tromsø Study: Fit Futures

Ole Andreas Nilsen,[1] Luai Awad Ahmed,[1] Anne Winther,[2] Tore Christoffersen,[1,3] Gyrd Thrane,[1] Elin Evensen,[4] Anne-Sofie Furberg,[5,8] Guri Grimnes,[6] Elaine Dennison,[7] and Nina Emaus[1]

[1]Department of Health and Care Sciences, The Arctic University of Norway, Tromsø, Norway
[2]Division of Neurosciences, Orthopedics and Rehabilitation Services, University Hospital of North Norway, Tromsø, Norway
[3]Department of Health and Care Sciences, Finnmark Hospital Trust, Alta, Norway
[4]Department of Clinical Research, University Hospital of North Norway, Tromsø, Norway, and Department of Health and Care Sciences, The Arctic University of Norway, Tromsø, Norway
[5]Department of Community Medicine, The Arctic University of Norway, Tromsø, Norway
[6]Division of Internal Medicine, University Hospital of North Norway, Tromsø, Norway, and Endocrine Research Group, Department of Clinical Medicine, The Arctic University of Norway, Tromsø, Norway
[7]MRC Lifecourse Epidemiology Unit, Southampton UK and Victoria University, Wellington, New Zealand
[8]Department of Microbiology and Infection Control, Division of Internal Medicine, University Hospital of North Norway, Tromsø, Norway

ABSTRACT

Determinants of bone acquisition in late adolescence and early adulthood are not well-described. This 2-year follow-up study explored the associations of body weight (BW), body mass index (BMI), and changes in weight status with adolescent bone accretion in a sample of 651 adolescents (355 girls and 296 boys) between 15 and 19 years of age from The Tromsø Study: Fit Futures. This Norwegian population-based cohort study was conducted from 2010 to 2011 and was repeated from 2012 to 2013. We measured femoral neck, total hip, and total body bone mineral content and areal bone mineral density (aBMD) by dual-energy X-ray absorptiometry. We measured height, BW, calculated BMI (kg/m^2), and collected information on lifestyle at both surveys. Mean BMI (SD) at baseline was 22.17 (3.76) and 22.18 (3.93) in girls and boys, respectively. Through multiple linear regression, baseline BW and BMI were positively associated with ΔaBMD over 2 years of follow-up at all skeletal sites in boys ($p < 0.05$), but not in girls. ΔBW and ΔBMI predicted ΔaBMD and ΔBMC in both sexes, but the strength of the associations was moderate. Individuals who lost weight during follow-up demonstrated a slowed progression of aBMD accretion compared with those gaining weight, but loss of BW or reduction of BMI during 2 years was not associated with net loss of aBMD. In conclusion, our results confirm that adequate BW for height in late adolescence is important for bone health. Associations between change in weight status and bone accretion during follow-up were moderate and unlikely to have any clinical implication on adolescents of normal weight. Underweight individuals, particularly boys, are at risk of not reaching optimal peak bone mass and could benefit from an increase in BMI. © 2019 The Authors. *JBMR Plus* is published by Wiley Periodicals, Inc. on behalf of the American Society for Bone and Mineral Research.

KEY WORDS: PEAK BONE MASS; BMI; ADOLESCENCE; GENERAL POPULATION STUDIES; DXA

Introduction

Osteoporosis is a major public health concern and a frequent cause of disability in Western societies.[1] Norway has one of the highest reported hip fracture incidences in the world.[2] Areal bone mineral density (aBMD) is a surrogate measure of bone strength and a strong predictor of fracture risk.[3] Although genetics explain a substantial proportion of the variance of an individual's bone mass, lifestyle factors influence skeletal dynamics particularly during growth. Adolescence is a critical period for bone accretion and attainment of peak bone mass, defined as the highest bone mass obtained in a lifetime.[4] Suboptimal acquisition of peak bone mass may

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Address correspondence to: OA Nilsen, Department of Health and Care Sciences, UiT The Arctic University of Norway. N-9037 Tromsø, Norway. E-mail: ole-andreas.nilsen@uit.no
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lead to increased risk of osteoporosis and fragility fractures in later life.[5,6]

It has long been established that there is an association between BW and bone mineral parameters in the adult population.[7] High BMI is generally considered to have an osteo-protective effect, while rapid loss of BW is associated with bone loss.[8,9] In childhood and adolescence, however, the relationship between weight status and bone accretion is more controversial. Both detrimental and protective effects of BW have been reported.[10–18] There are few studies with repeated measures exploring bone accretion and longitudinal relationships.[16] Obesity and overweight in childhood and adolescence are a growing concern worldwide with rising prevalence during the past decades.[19] In European countries, including Norway, there has been a shift in the BMI distribution, with an increase in BMI in the upper percentiles.[20] For health benefits, obese and overweight individuals are recommended to reduce their weight by approximately 10%. In older adults, evidence suggests that a weight reduction of that magnitude will induce a loss of bone of 1% to 2% and even up to 4% at highly trabecular sites such as the trochanter.[21]

Associations and interplay between anthropometric traits, aBMD levels, and bone accretion in late adolescence are not yet fully described and understood at a population level. The mechanisms behind the weight and bone relationship are not clear as both direct and indirect effects related to mechanical forces, nutrition, age, and hormonal status could be involved. The objectives of this 2-year follow-up population-based study were to explore the associations between baseline BW, baseline BMI, changes in BW (ΔBW), and changes in BMI (ΔBMI) on changes in bone mineral parameters in a Norwegian population from 15 to 19 years of age. We hypothesized that higher baseline BW and BMI, as well as ΔBW and ΔBMI would be positively associated with changes in bone parameters, and that negative ΔBW and ΔBMI could be detrimental to bone accrual in adolescents entering young adulthood.

Subjects and Methods

Subjects

Detailed information on the Fit Futures Study participants and study procedures has been published previously.[18] Briefly, the Fit Futures study, an expansion of the Tromsø study in Northern Norway,[22] invited all first year upper-secondary school students (15 to 17 years of age) in Tromsø and the neighboring municipalities to a comprehensive health survey in 2010 to 2011 (TFF1). In this initial survey, 1117 participants were invited and 1038 adolescents (508 girls and 530 boys) attended (attendance rate of 93%). Two years later, in 2012 to 2013, we invited all TFF1 participants and all third-year students in the same upper-secondary schools to a follow-up survey, Fit Futures 2 (TFF2), providing 688 repeated measures of aBMD (66% of the original cohort; Fig. 1). The Clinical Research Unit at the University Hospital of North Norway conducted both surveys during school days. The participants received information about the study in classrooms and all participants gave written informed consent at the study site. Participants younger than 16 years of age had to bring written consent from their guardians to take part in the survey. The data collection in TFF1 and TFF2 was approved by the Norwegian Data Protection Authority and the Regional Committee of Medical Research Ethics (REK nord) with project-specific approval for the present study (Ref. 2013/1459/REK nord).

Measurements

Femoral neck (FN), total hip (TH), and total body (TB) bone mineral content (BMC; g), bone area (BA; cm^2) and aBMD (g/cm^2) were measured by the same instrument (GE Lunar Prodigy; GE Lunar, Madison, WI, USA) by DXA and analyzed with enCORE pediatric software (GE Healthcare, Piscataway, NJ, USA)[23] in both TFF1 and TFF2. We used auto-analysis software and default region of interest, according to a standardized protocol. The primary outcome of the study was aBMD, but BMC and BA are reported to complement the understanding of bone accretion and growth. The precision of measurements expressed as coefficient of variation ([SD/mean] × 100) has previously been estimated to be 1.14% at the TH and 1.72% at the FN measured in vivo.[24] We used measurements of the left hip. In 15 cases the left hip data were erroneous or missing and the right hip data were reported for both TFF1 and TFF2. We measured height and BW to the nearest 0.1 cm and 0.1 kg on a Jenix DS 102 Stadiometer (Dong Sahn Jenix, Seoul, Korea), following standardized procedures. BMI was calculated as BW divided by height squared (kg/m^2), and participants were stratified into BMI quartiles. To explore if relationships changed with various BMI cut-off points, we also categorized participants into underweight, normal weight, overweight, or obese. Participants <18 years of age were stratified based on their sex- and age-specific BMI according to half-year cut-off points described by Cole and Lobstein.[25] To describe the crude impact of change-in-weight status on bone accretion, we dichotomized participants into BMI losers and BMI gainers.

Interviews and questionnaires

Information on ethnicity, the possibility of pregnancy (exclusion criterion for DXA), the presence of acute and chronic disease, and the use of medication and hormonal contraceptives was elicited by clinical interviews. We collected pubertal maturation information, perceived physical activity level, alcohol consumption, and tobacco use by electronic self-administered questionnaires. Pubertal status for girls was determined based on age at menarche and answers were categorized into "Early" (<12.5 years at menarche), "Intermediate" (12.5 to 13.9 years), or "Late" (>14 years) pubertal maturation. We used the Pubertal Developmental Scale (PDS) to assess pubertal maturation in boys. Secondary pubertal characteristics such as growth spurt, pubic hair growth, changes in voice, and facial hair growth were rated on a scale from 1 (Have Not Begun) to 4 (Completed), were summarized, and then were divided by 4. We categorized a score <2 as "Have Not Begun", 2 to 2.9 as "Barely Started", 3 to 3.9 as "Underway," and a score of 4 as "Completed."[26] Perceived physical activity level was assessed by a scale developed by Saltin and Grimby.[27] The participants were asked to grade leisure time physical activity an average week during the last year with four alternatives: sedentary activities only; moderate activity like walking, cycling, or exercise at least 4 hours per week; participation in recreational sports at least 4 hours per week; and participation in hard training/sports competitions several times a week. Questions on smoking and snuffing had three alternatives: Never, Sometimes, or Daily. We assessed the frequency of alcohol consumption with a scale from 1 to 5:

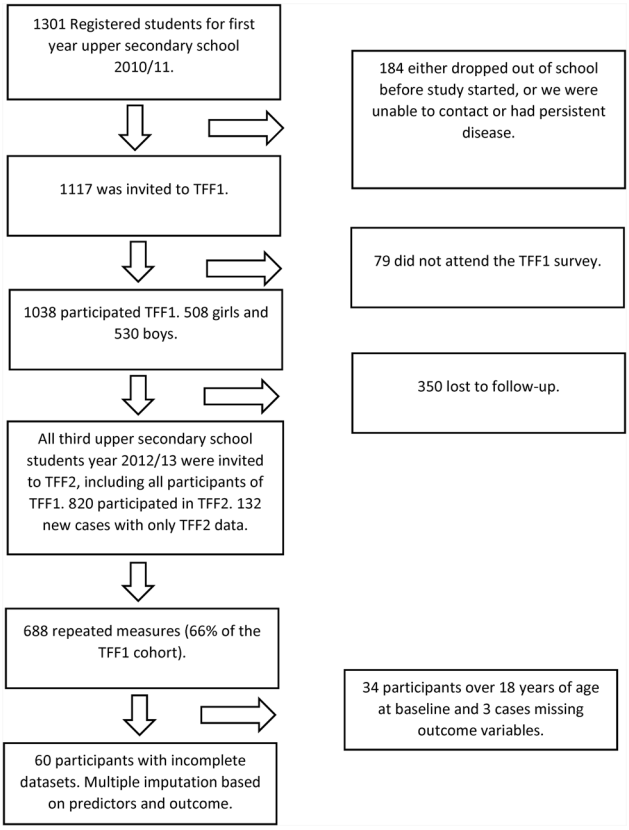


Fig. 1. Flowchart of participation in Fit Futures 1 (TFF1) 2010 to 2011 and Fit Futures 2 (TFF2) 2012 to 2013. The Tromsø Study, Fit Futures.

"Never," "Once per Month or Less," "2-4 Times per Month," "2-3 Times per Week," and "4 or More Times per Week." Answers on the use of medication known to affect bone, presence of diseases known to affect bone, hormonal contraceptive use, smoking, snuff use, and alcohol consumption were dichotomized into "Yes" and "No."

Statistical analyses

All analyses were sex-specific. Population characteristics are presented by BMI quartiles at baseline. Continuous variables are presented by means (SDs) and categorical variables by count (percentages). We compared BMI quartile groups by using one-way ANOVA with Bonferroni correction and χ^2 test. Welch's ANOVA with Games-Howell post hoc procedure was used if equal variances assumption was violated. We computed annual bone- and anthropometric-change variables to account for differences in time between baseline and follow-up measures when describing change and in crude comparisons of groups. Student's *t* test was used to compare BMI losers and BMI gainers.

Associations between the exposure variables baseline BW, baseline BMI, ΔBW, and ΔBMI and outcomes FN, TH, and TB ΔaBMD and ΔBMC during follow-up were assessed by multiple linear regression using the bone mineral follow-up score as outcome and baseline score as a covariate ($Y_2 = \beta 0 + \beta_1 Y_1 + \beta_2 X_{BW} + \beta_3 \ldots$). Initially we conducted a crude univariate analysis. We then compared the results using change-score analysis ($Y_2 - Y_1 = \beta 0 + \beta_1 X_{BW}$) and checking for consistency because baseline adjustments in change-score analysis may introduce bias.[28,29] All adjusted models included baseline anthropometric measures, time between measurements, pubertal maturation, and perceived baseline physical activity level. Other variables previously known to be of clinical importance like ethnicity, alcohol consumption, smoking, snuff use, diagnosis known to affect bone, medication known to affect bone (see Table 1, and hormonal contraceptive use (all baseline measures) were then added as covariates using a backwards elimination strategy where $p = 0.10$ were used as cut-off to enter or leave the model. Any covariate with $p \leq 0.10$ in a final model was included in all final models. Based on this procedure, alcohol consumption and diagnosis known to affect bone were excluded. We fitted separate models for baseline- and change-exposure variables. Models with ΔBW were adjusted for Δheight. We checked for confounding and plausible 2-way interactions related to age, pubertal maturation, and baseline weight versus weight change relationships. Because of statistical significance ($p < 0.05$) we added interaction terms BW * menarche age and BMI * menarche age in corresponding baseline ΔaBMD FN models in girls. In boys, a significant interaction between ΔBMI * BMI was detected and included in three ΔBMI models: FN ΔaBMD, FN ΔBMC, and TB ΔBMC; ΔBW * BW was added to the ΔBW TB ΔBMC model. Interactions were further explored and visualized by graphs.

Late introduction of the PDS questions in TFF1 may be the reason for a relatively high percentage of missing puberty values for boys: $n = 53$ (17.9%). Other missing covariates were

Table 1. Characteristics by BMI Quartiles at Baseline TFF1 (2010 to 2011). The Tromsø Study, Fit Futures

				BMI quartiles at baseline				
			Total	First quartile (n = 89)	Second quartile (n = 89)	Third quartile (n = 89)	Fourth quartile (n = 88)	p value
Girls (n = 355)	Age (years)		16.61 (0.387)	16.69 (0.44)	16.64 (0.36)	16.60 (0.38)	16.52 (0.35)	0.042
	Body height (cm)		165.03 (6.48)	165.77 (6.49)	165.92 (6.15)	164.65 (6.44)	163.95 (6.70)	0.127
	Body weight (kg)		60.37 (10.61)	51.31 (4.48)	56.59 (4.03)	60.87 (5.12)	72.97 (11.65)	<0.001
	BMI (kg/m^2)		22.17 (3.76)	18.65 (0.76)	20.54 (0.48)	22.42 (0.62)	27.13 (3.97)	<0.001
	FN aBMD (g/cm^2)		1.07 (0.12)	1.03 (0.11)	1.06 (0.13)	1.07 (0.13)	1.13 (0.11)	<0.001
	TH aBMD (g/cm^2)		1.06 (0.13)	1.02 (0.11)	1.05 (0.13)	1.06 (0.13)	1.12 (0.11)	<0.001
	TB aBMD (g/cm^2)		1.14 (0.08)	1.09 (0.06)	1.13 (0.07)	1.14 (0.07)	1.20 (0.06)	<0.001
	FN BMC (g)		4.91 (0.71)	4.62 (0.59)	4.82 (0.65)	4.89 (0.68)	5.31 (0.72)	<0.001
	TH BMC (g)		32.01 (4.84)	30.06 (4.31)	31.39 (4.48)	31.82 (4.51)	34.81 (4.84)	<0.001
	TB BMC (g)		2522.89 (387.38)	2256.31 (258.47)	2451.88 (266.57)	2528.10 (333.98)	2859.05 (407.61)	<0.001
	FN BA (cm^2)		4.59 (0.34)	4.50 (0.35)	4.57 (0.29)	4.59 (0.33)	4.73 (0.37)	<0.001
	TH BA (cm^2)		30.15 (2.33)	29.53 (2.26)	30.05 (1.83)	30.07 (2.40)	30.95 (2.58)	0.001
	TB BA (cm^2)		2207.37 (233.59)	2061.63 (165.65)	2170.54 (157.77)	2211.85 (207.55)	2384.14 (262.91)	<0.001
	Ethnicity	White	347 (97.8%)	84 (94.4%)	89 (100%)	88 (98.9%)	86 (97.7%)	0.068
		Others	8 (2.2%)	5 (5.6%)	0 (0%)	1 (1.1%)	2 (2.3%)	
	Menarche age (n = 348)	Early	110 (31.0%)	17 (19.3%)	22 (24.7%)	35 (40.2%)	36 (41.4%)	0.002
		Intermediate	165 (46.5%)	42 (47.7%)	48 (53.9%)	39 (44.8%)	39 (44.8%)	
		Late	73 (20.5%)	29 (33.0%)	19 (21.3%)	13 (14.9%)	12 (13.8%)	
	Physical activity at baseline	Sedentary	42 (12.0%)	17 (19.1%)	9 (10.0%)	7 (7.9%)	10 (11.2%)	0.054
		Moderate	141 (39.5%)	36 (40.4%)	26 (28.9%)	35 (39.3%)	44 (49.4%)	
		Sports	110 (30.8%)	22 (24.7%)	36 (40.0%)	28 (31.5%)	24 (27.0%)	
		Competition	63 (17.6%)	14 (15.7%)	19 (21.1%)	19 (21.3%)	11 (12.4%)	
	Alcohol (yes)		262 (73.2%)	58 (65.2%)	68 (75.6%)	72 (80.0%)	64 (71.9%)	0.160
	Smoking (yes)		68 (19.0%)	13 (14.6%)	15 (16.7%)	22 (24.4%)	18 (20.2%)	0.349
	Snuffing (yes)		108 (30.2%)	22 (24.7%)	24 (26.7%)	33 (36.7%)	29 (32.6%)	0.282
	Hormonal contraceptives use (yes)		118 (33.0%)	24 (27.0%)	32 (36.0%)	32 (36.0%)	30 (25.4%)	0.532
	Medication known to affect bone (yes)[a]		8 (2.2%)	1 (1.1%)	3 (3.4%)	3 (3.4%)	1 (1.1%)	0.646
	Diagnosis known to affect bone (yes)[b]		4 (1.1%)	0	1 (1.1%)	3 (3.4%)	0	0.199

			Total	First quartile (n = 74)	Second quartile (n = 74)	Third quartile (n = 74)	Fourth quartile (n = 74)	p value
Boys (n = 296)	Age (years)		16.60 (0.37)	16.50 (0.38)	16.63 (0.38)	16.67 (0.33)	16.61 (0.36)	0.034
	Body height (cm)		177.25 (6.52)	177.30 (6.45)	177.12 (7.05)	177.56 (6.56)	177.00 (6.10)	0.957
	Body weight (kg)		69.81 (13.68)	57.10 (5.16)	64.43 (5.49)	71.46 (5.49)	86.26 (14.11)	<0.001
	BMI (kg/m^2)		22.18 (3.93)	18.14 (.85)	20.50 (.60)	22.64 (.62)	27.45 (3.64)	<0.001
	FN aBMD (g/cm^2)		1.11 (0.15)	1.01 (0.11)	1.12 (0.14)	1.13 (0.13)	1.19 (0.16)	<0.001
	TH aBMD (g/cm^2)		1.12 (0.15)	1.02 (0.11)	1.12 (0.13)	1.15 (0.14)	1.20 (0.16)	<0.001
	TB aBMD (g/cm^2)		1.18 (0.10)	1.10 (0.08)	1.18 (0.08)	1.20 (0.08)	1.24 (0.09)	<0.001
	FN BMC (g)		5.99 (0.99)	5.32 (0.75)	6.01 (0.87)	6.12 (0.90)	6.53 (1.04)	<0.001
	TH BMC (g)		40.17 (6.64)	35.61 (5.20)	40.11 (5.76)	41.30 (6.09)	43.65 (6.79)	<0.001
	TB BMC (g)		2963.78 (469.83)	2556.57 (340.84)	2877.81 (330.39)	3084.27 (385.23)	3336.46 (432.67)	<0.001
	FN BA (cm^2)		5.38 (0.39)	5.29 (0.43)	5.37 (0.35)	5.40 (0.35)	5.48 (0.39)	0.024
	TH BA (cm^2)		35.73 (2.47)	34.71 (2.61)	35.69 (2.31)	36.03 (2.21)	36.48 (2.47)	<0.001
	TB BA (cm^2)		2496.46 (240.06)	2307.67 (189.40)	2443.49 (175.25)	2555.57 (201.59)	2679.11 (222.11)	<0.001
	Ethnicity	White	291 (98.3%)	74 (100%)	71 (95.9%)	74 (100%)	72 (97.3%)	

(Continues)

Table 1. (Continued)

		Total	First quartile (n = 74)	Second quartile (n = 74)	Third quartile (n = 74)	Fourth quartile (n = 74)	p value
	Others	5 (1.7%)	0 (0%)	3 (4.1%)	0 (0%)	2 (2.7%)	
Puberty development scale (n = 241)	Just started	22 (18.1%)	9 (16.7%)	12 (18.8%)	9 (14.3%)	14 (22.6%)	0.216
	Underway	177 (72.8%)	43 (79.6%)	49 (76.6%)	45 (71.4%)	40 (64.5%)	
	Completed	22 (9.1%)	2 (3.7%)	3 (4.7%)	9 (14.3%)	8 (12.9%)	
Physical activity at baseline (n = 293)	Sedentary	77 (26.3%)	24 (32.9%)	10 (13.5%)	14 (19.2%)	29 (39.7%)	0.004
	Moderate	75 (25.6%)	21 (28.8%)	22 (29.7%)	16 (21.9%)	16 (21.9%)	
	Sports	70 (24.2%)	17 (23.3%)	20 (27.0%)	17 (23.3%)	17 (23.3%)	
	Competition	62 (23.9%)	11 (15.1%)	22 (29.7%)	26 (35.6%)	11 (15.1%)	
Alcohol (yes)		195 (65.9%)	49 (66.2%)	41 (55.4%)	50 (67.6%)	55 (74.3%)	0.109
Smoking (yes)		62 (20.9%)	19 (25.7%)	9 (12.2%)	16 (21.6%)	18 (24.3%)	0.173
Snuffing (yes)		108 (36.5%)	30 (40.5%)	14 (18.9%)	30 (40.5%)	34 (45.9%)	0.003
Medication known to affect bone (yes)[a]		6 (2.0%)	1 (1.4%)	2 (2.7%)	1 (1.4%)	2 (2.7%)	>0.999
Diagnosis known to affect bone (yes)[b]		5 (1.7%)	1 (1.1%)	1 (1.1%)	2 (2.7%)	1 (1.1%)	>0.999

Continuous variables are described by mean (SD) and categorical by count (%).Cut-off points for BMI quartiles (kg/cm2) were 19.71, 21.43, and 23.48 in girls and 19.39, 21.56, and 23.77 in boys.

[a]Medication known to affect bone (ATC): D07A Plain corticosteroids, H03A Thyroid preparations, N03A Antiepileptic, R01AD Corticosteroids, R03BA Glucocorticoids (inhalants), and H02A Corticosteroids for systemic use.

[b]Diagnosis known to affect bone (according to the 10th revision of the International Statistical Classification of Diseases and Related Health Problems): E03 Hypothyroidism, E10 Diabetes type 1, F50.9 Eating disorders, K90.0 Celiac disease, and M13 Arthritis.

aBMD = Areal bone mineral density; BMC = bone mineral content; BA = bone area; FN = femoral neck; TH = total hip; TB = total body; ATC = Anatomical Therapeutic Chemical.

menarche age in seven girls and physical activity in one girl and three boys. Multiple imputations based on predictors and outcome variables were performed to predict missing values. We assumed missing at random and 20 imputations were conducted,[30] and we report pooled estimates. Normal distribution, linearity, homogeneity, and outliers were explored by residual analysis. In girls, two outliers were excluded in TH ΔaBMD: one in FN ΔaBMD and one in TH ΔBMC models. We used weighted least square regression in all TB ΔBMC models in girls to account for heteroscedasticity. Significance level was set to p = 0.05 and all procedures were performed in IBM SPSS Statistics for Windows, version 24 (IBM Corp., Armonk, NY, USA). Figures were made in RStudio (RStudio, Boston, MA, USA; (http://www.rstudio.com/)

Results

Descriptives

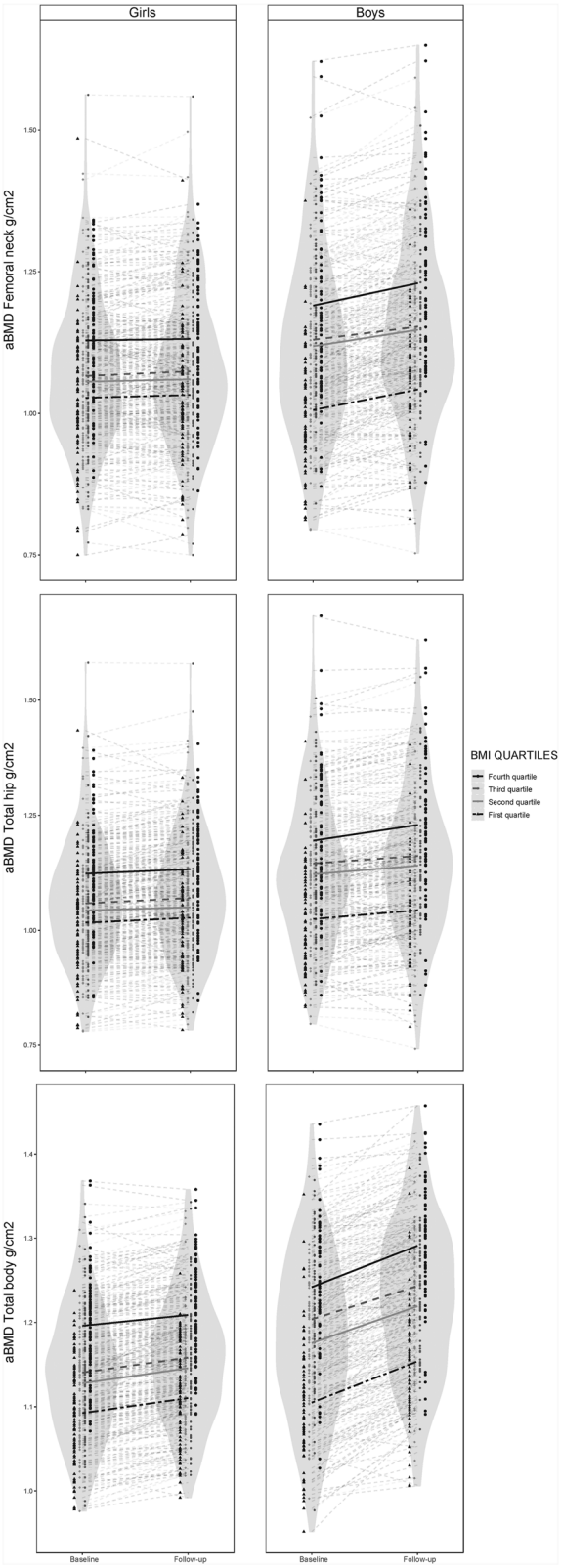
We included 651 adolescents with repeated measurements in the analyses, 355 girls and 296 boys (45.2% boys). At baseline, mean age was 16.6 years (range, 15.7 to 17.9), and 18.6 years (range, 17.8 to 20.1) at follow-up. Average follow-up time was 1.94 years (SD 0.2). Table 1 displays the baseline characteristics according to BMI quartile groups. In girls, mean group BMI for first to fourth quartile were 18.65, 20.54, 22.42, and 27.13 kg/m^2, respectively. In boys, means were 18.14, 20.50, 22.64, and 27.45 kg/m^2, respectively.

One-way ANOVA analyses showed that cross-sectional anthropometric, aBMD, and BMC measures differed significantly with a positive linear trend between BMI quartiles at baseline, except body height. These cross-sectional differences persisted at follow-up 2 years later (not shown). In girls, menarche age differed significantly with higher prevalence of early menarche at the two upper BMI quartiles compared with the bottom quartile (p = 0.002). In boys, physical activity at baseline differed significantly (p = 0.004) with a higher prevalence of sedentary behavior for the upper quartile (39.7%) compared with the other quartiles, and there was a low proportion of snuff users in the second quartile compared with the three other groups (p = 0.003).

Among girls, 5.9% of participants were classified as underweight, 75.4% normal weight, 14.0% overweight, and 4.7% obese according to Cole's weight classification at baseline with a mean group BMI of 17.6, 21.9, 26.2, and 33.9 kg/m^2, respectively. Among boys, 8.4% of participants was classified as underweight, 70.6% normal weight, 14.5% overweight, and 6.4% as obese. Mean group BMI in boys were 17.2, 21.0, 26.2, and 32.5 kg/m^2, respectively. Proportions in the two upper categories increased during follow-up. In girls, the prevalence of overweight and obesity combined had increased to 20.6% in 2 years. In boys, the prevalence of overweight and obesity combined increased to 28% at TFF2 (data not shown).

In girls, mean annual BW and BMI change was 1.38 kg (95% confidence interval [CI], 1.12 to 1.64) and 0.41 kg/m^2 (95% CI, 0.31 to 0.50). Boys gained 2.70 kg (95% CI, 2.35 to 3.04) and 0.61 kg/m^2 (95% CI, 0.51 to 0.72), respectively. Eighty-eight girls (24.6%) and 48 boys (16.2%) lost BW with an average annual loss of –1.60 (95% CI, –1.92 to –1.28) and –1.97 (95% CI, –2.43 to –1.51) kg. One-hundred eleven girls (31.3%) and 62 boys (20.9%) reduced their BMI during follow-up, with a mean annual decrease of –0.56 (95% CI, –0.66 to –0.46) and –0.66 (95% CI, –0.81 to –0.51) kg/m^2. We observed a clear difference in longitudinal growth between girls and boys. In girls, 280 (78.9%) of the participants had an

increment in height between measurements with an annual mean of 0.053 cm (95% CI, 0.049 to 0.056). Almost all the boys (93.2%, n = 276) grew taller during the 2 years of follow-up. Annual mean change was 1.024 cm (95% CI, 0.928 to 1.120).



Cross-sectional measures and the individual aBMD trajectories from TFF1 to TFF2 and unadjusted means within baseline BMI quartiles are illustrated in Fig. 2. Post hoc analysis showed that, among boys, the first quartile had significantly lower, and the fourth quartile significantly higher FN, TH, and TB aBMD than the other quartiles at both time points ($p < 0.05$). There were no significant differences in aBMD status between second and third quartiles in any of the three skeletal sites, neither at baseline nor at follow-up. In girls, the pattern appeared similar to boys, but less polarized in the lower BMI quartiles. The aBMD levels in girls in the first quartile did not differ significantly from the two middle quartiles at the femoral sites.

When participants were stratified into BMI categories, the relationships slightly changed. Figure 3 indicates that although not statistically significant, and unlike the girls, boys in the obese category had lower mean FN, TH, and TB aBMD at both measure points compared with their overweight peers. Boys classified as underweight had significantly lower aBMD at baseline compared with those with normal weight (FN: $p = 0.001$, TH: $p = 0.005$, TB: $p < 0.001$) and this pattern persisted during the 2 years of follow-up in crude analyses.

Body weight, body mass index, and bone accretion

Changes in anthropometry, ΔaBMD, and ΔBMC during follow-up according to baseline BMI quartiles are presented in Table 2. In crude comparisons of quartiles, no statistically significant differences were found, except ΔBW, ΔBMI, and ΔTB BMC in girls. The first quartile gained more weight compared with the second quartile, and accumulated more total body bone than the fourth quartile.

Figure 4 depicts mean ΔaBMD (Fig. 4*A*) and ΔBMC (Fig. 4*B*) among BMI losers and BMI gainers between TFF1 and TFF2. Reduction of BMI seemed to induce a slower bone accretion rate, especially in boys, but no mean bone loss was observed in any BMI loser group in either girls or boys. Among girls, statistically significant differences between the two groups were found only at TB ΔBMC ($p < 0.001$). Among boys, TH ΔaBMD ($p = 0.027$), TB ΔaBMD ($p = 0.011$), FN ΔBMC ($p = 0.033$), TH ΔBMC ($p < 0.001$), and TB ΔBMC ($p < 0.001$) were significant. The same pattern was observed with loss of BW. In boys, the BW loser group ($n = 48$) had a mean annual increment in TH aBMD of 0.006 g/cm^2 (95% CI, 0.000 to 0.012); the BW gainers had a mean of 0.012 g/cm^2 (95% CI, 0.010 to 0.015; not shown).

The crude and adjusted associations from multiple linear regression models between baseline BW, baseline BMI, ΔBW, ΔBMI, and ΔaBMD and ΔBMC are presented in Table 3. In girls, no associations between baseline measures and ΔaBMD were identified, but both baseline BW ($p = 0.009$) and baseline BMI

Fig. 2. Femoral neck-, total hip-, and total-body aBMD in girls and boys from TFF1 (2010 to 2011) to TFF2 (2012 to 2013). Individual measures and group mean according to BMI quartiles at baseline. Girls, n = 355. Boys, n = 296. The Tromsø Study, Fit Futures. In girls, cut-off points for BMI quartiles were 19.7, 21.4, and 23.5 and in boys 19.4, 21.6, and 23.8, respectively. The grey area (violin plot) shows the full population distribution at TFF1 and TFF2 in both girls and boys. The points specify each individual measurement and the thin dotted lines show participants individual accretion during follow-up. The thick lines indicate the baseline BMI quartile group mean aBMD accretion between measurements. aBMD = Areal bone mineral density; BMI = body mass index (kg/m^2).

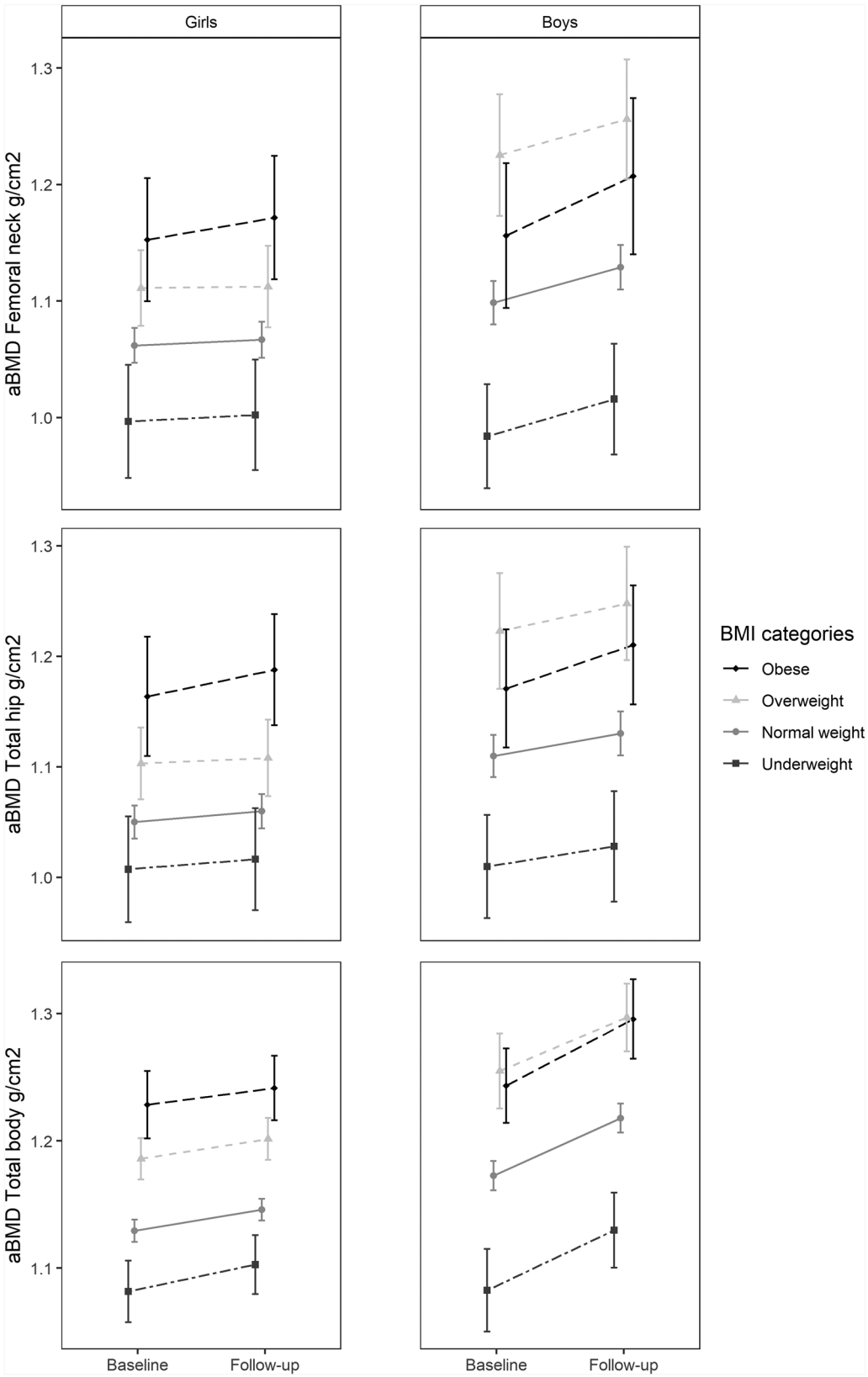


Fig. 3. Mean aBMD accretion of femoral neck, total hip and total body aBMD in girls and boys between baseline survey TFF1 (2010 to 2011) and the follow-up survey TFF2 (2012 to 2013) according to baseline BMI categories underweight, normal weight, overweight, and obese. The Tromsø Study, Fit Futures. Girls, n = 355. Boys, n = 296. In girls, the BMI intervals for baseline BMI categories were: underweight: 16.2 to 18.2, normal weight: 18.1 to 24.7, overweight: 24.5 to 29.1, and obese: 29.8 to 41.2 (kg/cm^2). In boys, the intervals were 16.2 to 17.8, 17.7 to 24.2, 24.2 to 28.9, and 29.6 to 40.3 (kg/cm^2), respectively. Error bars = 95% confidence interval. aBMD = Areal bone mineral density; BMI = body mass index (kg/cm^2).

Table 2. Annual change in body height (cm), body weight (kg), BMI (kg/m^2), aBMD (g/cm^2) and BMC (g) between TFF1 (2010-2011) and TFF2 (2012-2013) by BMI quartiles at baseline. The Tromsø Study, Fit Futures

			BMI quartiles at baseline				
		Total	First quartile	Second quartile	Third quartile	Fourth quartile	p-value
Girls	Δ Body height	0.365 (0.455)	0.419 (0.521)	0.389 (0.388)	0.325 (0.435)	0.326 (0.455)	0.417
(n = 355)	Δ Body weight	1.383 (2.501)	1.928 (1.718)[2]	0.934 (1.944)[1]	1.275 (2.238)	1.394 (3.614)	0.004
	Δ BMI	0.406 (0.910)	0.608 (0.656)[2]	0.243 (0.699)[1]	0.381 (0.852)	0.392 (1.280)	0.005
	Δ FN aBMD	0.003 (0.019)	0.003 (0.018)	0.003 (0.019)	0.005 (0.020)	0.002 (0.018)	0.755
	Δ TH aBMD	0.005 (0.017)	0.006 (0.017)	0.004 (0.018)	0.006 (0.017)	0.005 (0.016)	0.809
	Δ TB aBMD	0.009 (0.010)	0.009 (0.010)	0.010 (0.009)	0.009 (0.011)	0.006 (0.010)	0.094
	Δ FN BMC	0.014 (0.095)	0.011 (0.093)	0.017 (0.093)	0.017 (0.096)	0.013 (0.099)	0.970
	Δ TH BMC	0.180 (0.592)	0.241 (0.605)	0.116 (0.557)	0.171 (0.626)	0.193 (0.580)	0.563
	Δ TB BMC	39.609 (60.362)	55.290 (37.087)[4]	32.229 (50.509)	45.379 (52.165)	25.379 (86.922)[1]	0.001
Boys	Δ Body height	0.929 (0.867)	1.076 (1.011)	0.896 (0.619)	0.864 (1.103)	0.882 (0.624)	0.414
(n = 296)	Δ Body weight	2.697 (3.022)	2.928 (2.332)	2.974 (2.413)	2.661 (3.370)	2.224 (3.732)	0.481
	Δ BMI	0.614 (0.950)	0.692 (0.718)	0.713 (0.736)	0.629 (1.082)	0.424 (1.170)	0.315
	Δ FN aBMD	0.16 (0.027)	0.018 (0.025)	0.015 (0.026)	0.013 (0.028)	0.020 (0.028)	0.402
	Δ TH aBMD	0.012 (0.022)	0.010 (0.022)	0.010 (0.023)	0.009 (0.023)	0.017 (0.022)	0.093
	Δ TB aBMD	0.023 (0.015)	0.024 (0.016)	0.022 (0.015)	0.021 (0.015)	0.024 (0.016)	0.475
	Δ FN BMC	0.100 (0.176)	0.107 (0.173)	0.089 (0.175)	0.077 (0.176)	0.129 (0.180)	0.308
	Δ TH BMC	0.566 (1.072)	0.514 (1.067)	0.527 (1.177)	0.440 (1.029)	0.783 (0.997)	0.229
	Δ TB BMC	118.818 (77.247)	121.371 (67.240)	121.005 (69.577)	118. 124 (81.233)	114.773 (90.133)	0.951

aBMD =Areal bone mineral density (g/cm^2), BMC = Bone mineral content (g), FN = Femoral neck, TH = Total hip, TB = Total body, BMI = Body mass index (kg/cm^2), body weight in kg, Δ = change. Cut-offs points for BMI quartiles were 19.71, 21.43, 23.48 (kg/m^2) in girls and 19.39, 21.56, 23.77 (kg/m^2) in boys. Average follow-up time was 1.94 years (SD 0.2).[1234] Significantly different from specified quartile ($p < 0.05$) analysed using bonferroni post-hoc test for multiple comparisons.

($p = 0.021$) were significantly associated with ΔBMC in the adjusted TH models. In boys, baseline BW and BMI were statistically significant predictors of both ΔaBMD and ΔBMC in most models. Exceptions were crude FN ΔaBMD/ΔBMC and TB ΔBMC. ΔBW and ΔBMI had a consistent positive association with both ΔaBMD and ΔBMC in all adjusted models, except ΔBMI ΔaBMD TH ($p = 0.086$). The influence on ΔaBMD was strongest at femoral sites in boys, but overall changes in aBMD were moderate considering the size of the units of exposure. A baseline BMI difference of 1 SD (3.93 kg/m^2) was associated with a 0.008 g/cm^2 difference in TH ΔaBMD over 2 years ($p = 0.002$), whereas 1 SD ΔBMI (1.89 kg/m^2) during follow-up was associated with 0.004 g/cm^2 ΔaBMD ($p = 0.086$). Statistically significant interactions were detected in six models. Pubertal maturation moderated the relationship of baseline BW/BMI and FN ΔaBMD in girls, whereas initial BW and BMI appeared to influence some of the change in weight–bone accretion associations in FN and TB among boys. The relationships between bone accretion and weight change were strongest among boys with low BMI/BW at baseline (Table 3, Fig. 5*A* and Fig. 5*B*).

Discussion

In this population-based study we explored the associations between BW, BMI, ΔBW, and ΔBMI with changes in bone parameters in adolescents entering young adulthood. Underweight boys had significantly lower mean aBMD at baseline and this disadvantage persisted during 2 years of follow-up. Change in BW and BMI appeared to be a significant predictor of aBMD change for both girls and boys in the adjusted models, but the increments of aBMD for each unit change in exposure were relatively modest. Findings suggest that the influence of weight change might be strongest among boys with low BMI. Loss of BW or reduction of BMI was not associated with net loss of aBMD; however, our results indicate that the bone accretion rate slowed down whenever weight was lost or BMI reduced during follow-up in both sexes. In the present study, more than one of five adolescents was classified as overweight or obese at baseline; the prevalence increased during follow-up for both girls and boys.

The results supported our initial hypothesis with a few exceptions. In girls, the influence of baseline weight status on ΔaBMD was limited compared with the results in boys. This may be caused by gender differences in maturation. Cessation of longitudinal growth in girls and strong genetic control reduce the accumulation of bone mass. Previously published results indicate that girls reach a femoral aBMD plateau between 17 and 19 years of age.[18] The influence of baseline BW and BMI may therefore be less in girls in this age interval because adaptation to mechanical loading is greater in a growing skeleton.[31]

A positive cross-sectional association between BMI and aBMD and a positive association between baseline BW and increased *Z*-score in femoral sites over 2 years in boys have previously been shown in the Fit Futures cohort.[18,32] In the present study, we report that cross-sectional associations between BMI and aBMD were still present at TFF2 in both girls and boys 2 years later. Our findings are in accordance with a recent meta-analysis and systematic review by Van Leeuwen and colleagues.[16] They included 27 observational studies on the relationship between BW and bone mineral parameters in participants between 2 to 18 years of age and concluded that overweight and obese individuals had significantly higher aBMD and BMC than counterparts with normal BW. However, only one longitudinal study exploring the long-term consequences of childhood obesity was included in the meta-analysis. Threshold effects of BMI´s positive influence on bone have been previously reported.[33,34] Although nonsignificant and based on a small

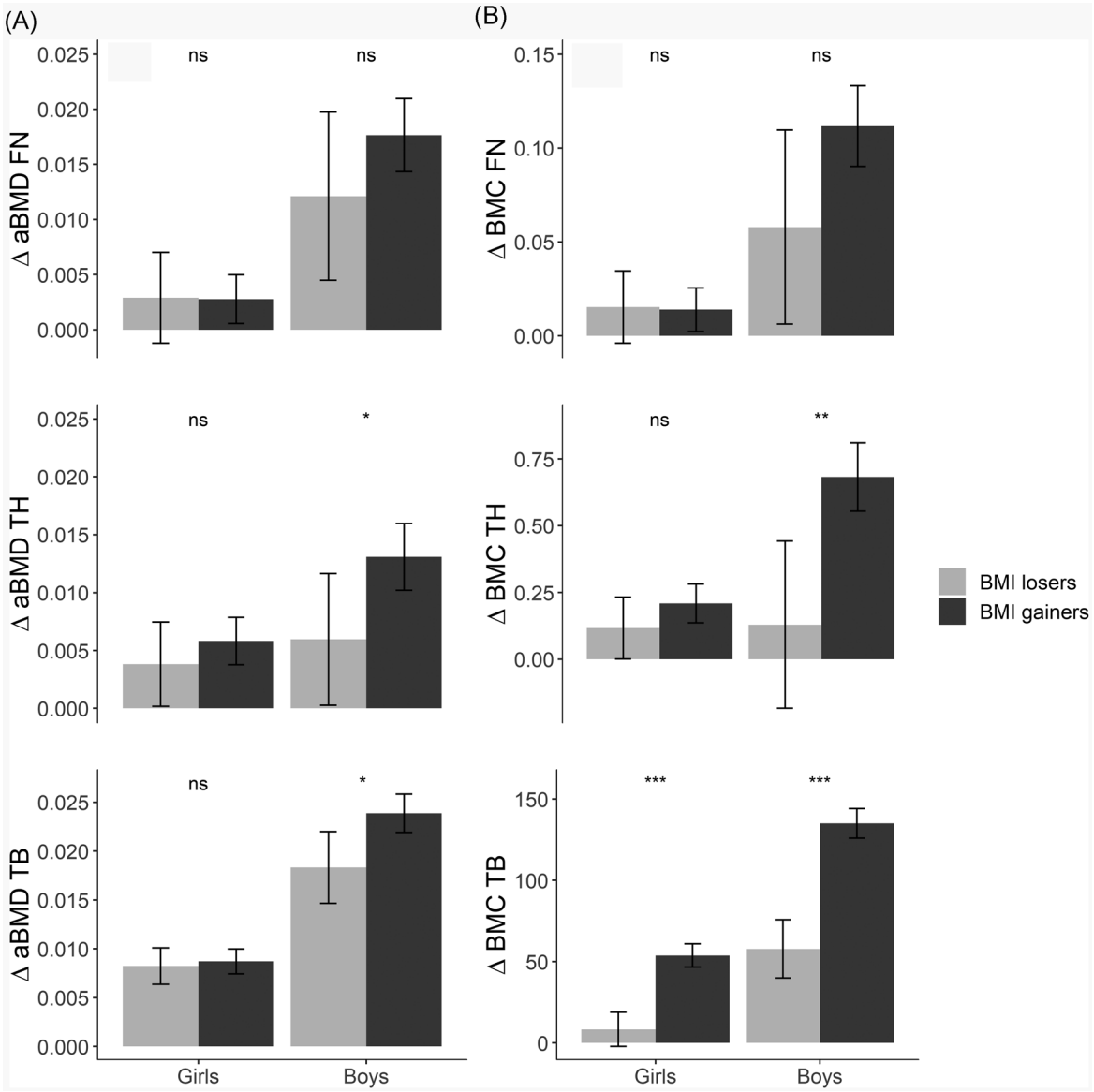


Fig. 4. Mean annual (*A*) aBMD and (*B*) BMC change in BMI losers and BMI gainers between baseline survey TFF1 (2010 to 2011) and the follow-up survey TFF2 (2012 to 2013). The Tromsø Study, Fit Futures. Girls, $n = 355$. Boys, $n = 296$. BMI loser girls: $n = 111$, BMI losers boys: $n = 62$. FN = Femoral neck; TH = total hip; TB = total body; ΔaBMD = change in areal bone mineral density (g/cm^2); ΔBMC = change in bone mineral content (g); BMI = body mass index (kg/cm^2). Error bars = 95% confidence interval. Two-tailed *t*-test for differences in mean: ns: $p > 0.05$, $^*p \leq 0.05$, $^{**}p \leq 0.01$, $^{***}p \leq 0.001$.

number of subjects, we observed that adolescent boys classified as overweight had the highest mean aBMD, higher than those classified as obese. This pattern was not observed when participants were stratified into BMI quartiles. The mean BMI was higher in the obese category (32.5 kg/m^2) than the fourth BMI quartile (27.1 kg/m^2), representing the tail of the distribution. In girls, the associations between baseline BMI categories and measured bone traits were positive and had a linear trend.

Change in body weight and BMI and accretion of aBMD

Bone loss during weight reduction is well-documented in older individuals, but not yet demonstrated in younger populations.[21] We found no net loss of aBMD or BMC in participants losing BW or reducing their BMI during follow-up. However, mean annual BMI reduction was modest (−0.56 kg/cm^2 among girls and −0.66 kg/cm^2 among boys) over 2 years in our study. To investigate more extreme cases of weight loss, an elaborate analysis stratifying ΔBMI in deciles was conducted (within 10th percentile, mean annual ΔBMI of −1.16 kg/m^2 in both girls and boys), but a significant loss of aBMD was still not detected (not shown). The association between weight loss and loss of bone is more consistent in older compared with younger individuals.[35] This may be linked to relatively better maintained muscle function in the younger age groups.[21] There is a strong relationship between lean mass and bone, and healthy adolescents are less vulnerable to loss of muscle function during weight reduction compared with older peers. Furthermore, older people may be more prone

Table 3. Adjusted associations between baseline and changes in weight parameters and femoral bone development during two year follow-up. The Tromsø Study, Fit Futures

			FN				TH				TB			
			Crude		Adjusted		Crude		Adjusted		Crude		Adjusted[¤]	
			β	p	β	p	β	p	β	p	β	p	β	p
Girls n = 355	ΔaBMD	Body weight	.003	.099	.001[*]	.669	.003	.116	.003	.189	.002	.184	.000	.971
		Body weight x menarche age			−.003	.013								
		BMI	.001	.546	.001[*]	.779	.002	.335	.002	.200	.000	.925	−.001	.607
		BMI x menarche age			−.003	.009								
		Δ Body weight	.004	.057	.002	.002	.005	.005	.005	.004	.002	.026	.002	.083
		Δ BMI	.001	.560	.001	.001	.004	.030	.004	.016	.002	.110	.001	.169
	ΔBMC	Body weight	.024	.029	.019	.105	.171	.013	.182	.009	9.891	.294	7.074	.461
		BMI	.009	.378	.010	.339	.112	.076	.148	.021	−3.405	.642	−1.900	.803
		Δ Body weight	.026	.008	.024	.009	.221	<.001	.218	<.000	64.494	<.001	66.417	<.000
		Δ BMI	.015	.125	.021	.025	.181	.002	.287	.001	60.323	<.001	63.387	<.000

			FN				TH				TB			
			Crude		Adjusted		Crude		Adjusted		Crude		Adjusted	
			β	p	β	p	β	p	β	p	β	p	β	p
Boys n = 296	ΔaBMD	Body weight	.008	.005	.009	.009	.008	.005	.009	.002	.006	.002	.006	.005
		BMI	.006	.076	.008	.008	.006	.021	.008	.002	.004	.024	.005	.007
		Δ Body weight	.007	.015	.004	.004	.008	.002	.005	.023	.008	<.001	.007	.003
		Δ BMI	.003	.333	.005[#]	.081	.004	.083	.004	.086	.006	.001	.006	.001
		Δ BMI x BMI			−.008	.004								
	ΔBMC	Body weight	.059	.009	.078	.001	.072	.023	.374	.005	33.515	.008	34.190	.007
		BMI	.051	.017	.070	.001	.268	.043	.399	<.000	16.358	.130	18.815	.085
		Δ Body weight	.064	.001	.040	.030	.548	<.001	.328	.005	93.669	<.001	87.503[§]	.000
		Δ Body weight x body weight											−19.109	.004
		Δ BMI	.031	.120	.049[#]	.017	.347	.005	.452	.002	77.863	<.001	84.189[#]	.000
		Δ BMI x BMI			−.056	.003							−24.348	.001

All β coefficients are per SD change in exposure. BMC = Bone mineral content (g), FN = Femoral neck, TH = Total hip, TB= Total body, BMI= Body mass index (kg/m^2), body weight in kg. Δ = change. adjusted models included age, sexual maturation, physical activity level, baseline aBMD or BMC measurement, time between measurements, ethnicity, use of medication known to affect bone, hormonal contraceptives use (girls), snuff use and smoking. In girls, one outlier in FN ΔaBMD (n = 354) models was excluded, two in TH ΔaBMD (n = 353) and one in TH ΔBMC models (n = 354). All baseline body weight models were adjusted for baseline height. ΔBody weight models were adjusted for baseline height and Δ height, whereas Δ BMI models adjusted for baseline BMI. Multiple imputation were conducted based on predictors and outcome variables in the adjusted models and pooled estimates are shown. ¤ Weighted least square regression (n = 348 because imputation were not used). *The effect of weight and BMI should be measured as (β1 + β3 (menarche age)), #The effect of Δ BMI should be measured as (β1 + β3 (BMI)), § The effect of Δ body weight should be measured as (β1 + β3 (body weight)). All interactions are based on mean-centered variables and visually explored in Figure 5.

to bone loss because of reduced efficiency in calcium absorption with age.[36]

The determinants of bone acquisition in the period of late adolescence to early adulthood are understudied,[4] and there are a limited number of studies of weight change and bone in a comparable population. Most studies are among pre-, peri-, and postmenopausal women, in relation to weight-reduction interventions, eating disorders, use of medications, or bariatric surgery.[21,37,38] Studies on anorexia nervosa in adolescence are not directly comparable, but longitudinal studies of weight gain and restoration of BW show significant, although slow, improvement and normalization of aBMD levels.[39] In a recent study, extensive BMI gain during puberty was associated with lower increments in aBMD.[40] Exploring the effect of weight change on bone mass in obese female adolescents, Rourke and colleagues[41] found no bone loss, but concluded that reduction of BW induced a reduced bone growth rate over 12-month follow-up—results that are comparable to our findings.

The effect of weight reduction on bone depends on whether it is voluntary or involuntary, the rate of change, age, sex, and initial weight.[37] In the current study, we had no information on the reason for our participants' BW reduction, whether it was based on dieting, disease/illness, or natural fluctuations. Normally, adults' BW fluctuates by >0.25 kg/year, but in adolescence BW may be more unstable.[42] Furthermore, we have no information on when during the 2-year follow-up the weight change occurred. The adaptive response delay of bones makes interpretations harder. Changes in weight precede skeletal adaptation to mechanical loads; the bone mass adaptation rate seems to depend on direction and magnitude as changes are more rapid during unloading than reloading.[8] Bone adaptation to weight change has also been shown to be

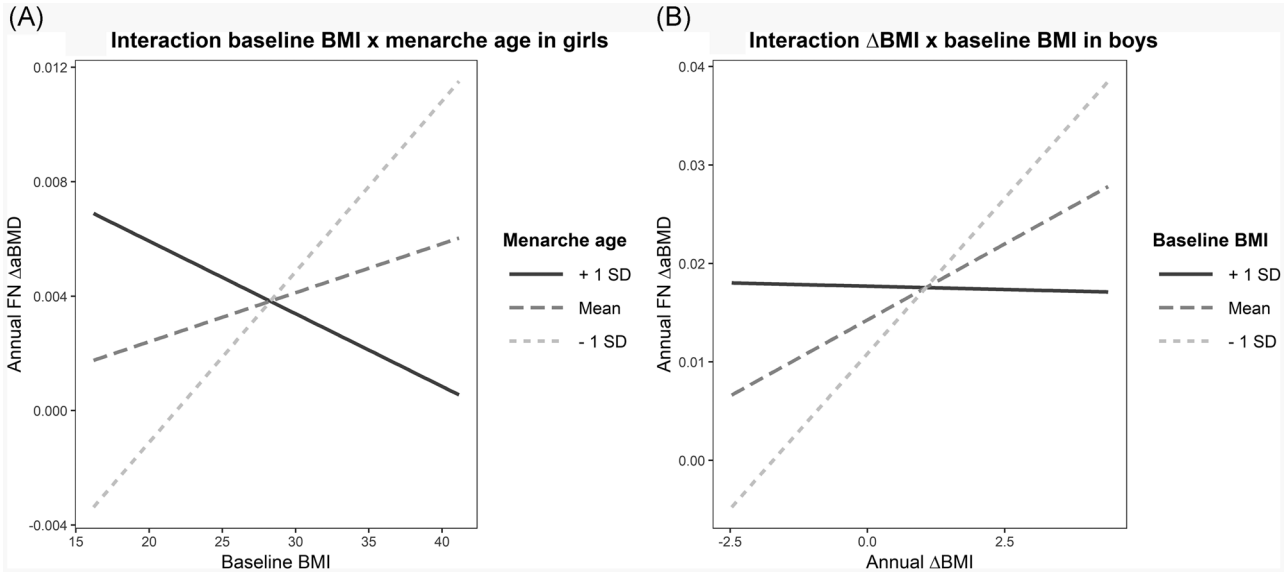


Fig. 5. Visualization of interactions (*A*) baseline BMI and menarche age in girls and (*B*) baseline BMI and ΔBMI in boys in femoral neck ΔaBMD regression models. The Tromsø Study, Fit Futures. Girls: $n = 354$. Boys: $n = 296$. Interaction plots show unadjusted relationships from linear regression models, but the interactions persisted after adjustments of relevant confounders. Menarche age: mean (SD) =12.98 (1.19), baseline BMI in boys: mean (SD) = 22.18 (3.93). aBMD = Areal bone mineral density (g/cm^2); BMI= body mass index (kg/m^2); Δ = change.

modified by exercise, nutrition, and medication.[21] Compared with high initial body weight, leaner individuals have been demonstrated to suffer greater bone loss during weight reduction.[21] We detected a statistically significant interaction between baseline BMI and ΔBMI in ΔaBMD FN model in boys indicating that the relationship between ΔBMI and bone accretion were strongest in boys with low BMI at baseline. In a crude analysis, this could very well be participants in the first quartile "catching up" based on age and pubertal maturation, but the relationship persisted after adjustments and the interaction was still present in the fully adjusted model. This interaction is potentially interesting; however, associations and relationships need to be tested and confirmed in other cohorts.

BMI reflects both muscle and adiposity; the mechanisms behind the relationship between weight status and bone are complex and multifactorial. Excess weight may have both negative and positive influences on bone health through different mechanisms. The process of bone modeling is sensitive to mechanical loading: It has been stated that high BW improves bone mineralization by increasing the forces applied on weight-bearing bones.[43] This effect has similarities to the positive effect of weight-bearing physical activities on bones.[16,44] Both weight-bearing activity and excess BW could lead to more lean mass. Greater lean mass, in addition to compressive force, produces increased tensile force on bone load and muscles produce the largest physiologic force on bone.[45] Results in our study indicate that, in girls, weight-based (and weight-bearing-based) interventions to maximize the genetic potential of peak bone mass at femoral sites should be implemented before the age of 15 years to be most effective. This is in agreement with studies indicating that prepuberty is the best time to change bone mass trajectory.[46]

On the other hand, weight-bearing activity is essential during growth and excess BW may be associated with sedentary behavior (in the present study, 39.7% of the boys reported to be sedentary in the upper BMI quartile). In addition to the mechanical-loading factors, adipose tissue may exert an impact on bone homeostasis and bone turnover through various adipokines like leptin and estrogen.[15] Mechanisms behind the correlation between changes in weight and bone changes in older populations are proposed to be related to estrogen bioavailability or/and decreased calcium intake. Studies showing a reduction of BMC in the distal forearm during dietary weight reduction suggest hormonal aspects are involved, not just gravity and a response to weight-bearing related forces.[47] There is also evidence suggesting that obesity may influence the timing of puberty. Dimitri and colleagues[10] highlight the effect of sex-related changes in body composition when studying relationships between bone and body size. Obese children reach peak height velocity earlier than age-matched lean children do, and late menarche is a determinant of lower aBMD and a known risk factor for fractures later in life.[48] Thus, an early menarche in obese girls may have a long term osteoprotective effect. In the present study, menarche age moderated the baseline BMI versus FN ΔaBMD relationship. Among girls with self-reported late menarche age, BMI appeared to be negatively correlated with FN ΔaBMD during follow-up. This interaction was, however, partly driven by a few individuals with baseline BMI >35 with considerable regression line leverage, and the statistical significance of interaction attenuated ($p = 0.083$) when these participants were excluded in a sensitivity analysis.

Strengths and limitations

The population-based design and repeated measures from a well-described representative sample of both sexes from different municipalities gave strengths to the present study. The sample size provided an opportunity to analyze the results in smaller subsamples, and explorations of the tails of the

distribution are of clinical interest. Using a dedicated research unit at the University Hospital of North Norway ensured the high quality of the data acquisition. We used the same densitometer through both surveys, with continuous validations following a standardized common protocol. The main limitations of this study were the short follow-up period of 2 years and that individuals were only measured twice. Short follow-up periods increase the risk of being obscured by variability in DXA measurements. On the other hand, the recommended minimum interval between DXA scans is 6 to 12 months.[49] Difference scores with two time points have limitations when exploring growth and development processes because the shape of the trajectory is unknown and additional measures would be preferred.[50] There are different approaches when assessing correlates of change between two time points. Difference-score as outcome ($Y_2 - Y_1$) and follow-up measurement (Y_2) as outcome using baseline (Y_1) as a covariate are two frequently used methods. Authors recommend a comparison of methods for agreement because in some situations these two approaches can lead to a different conclusion in nonrandomized studies based on the statistical phenomenon regression to the mean and Lord's paradox.[29,51] We found agreement in femoral ΔaBMD models, but discrepancy in some of the TB and BMC associations (Supplemental Table S2). Thus, results from the multiple regression model concerning some of the TB and BMC in this study should be interpreted with caution. Nevertheless, discrepancies may also be explained by the fact that dissimilarities in models as difference-scores without baseline adjustment fail to take the initial aBMD or BMC levels into account, consequently addressing slightly different concepts.

The 2D areal DXA measures have a tendency of overestimating BMC of larger bone because wider bones are also thicker; hence, the interpretation of measures of growing skeletons must be done with caution because of this size dependency.[52] This concern especially applies to our male participants still experiencing longitudinal growth. Shape, body habitus, and changes in body composition may affect DXA measurements; it has been suggested that DXA may not be a valid technique for evaluating bone/weight associations.[53] The impact of thickness of body tissue overlaying the measured area could be a concern in longitudinal studies of the effect of BW changes.[54,55] However, this mainly applies to lateral scans not performed in this study[56,57] and weight loss <6 kg has been shown to have limited influence on DXA aBMD measures.[37] Dietary intake information such as calcium intake and vitamin D levels may play a role in bone accretion. Unfortunately, information on nutrition was not available in The Fit Future study. Changes during follow-up in some of the control variables, such as increased proportions of smokers and snuff users, make the interpretations of associations harder (Supplemental Table S1). Nonparticipation and loss to follow-up bias could be a problem. With the high attendance rate of 93% of those invited at baseline, the nonparticipation exposition is limited. Drop-out analysis showed a higher proportion of boys, smokers, snuff users, and consumers of alcohol (girls) among the 32% lost at follow-up compared with those who participated in both surveys. Girls lost at follow-up had a moderately higher mean baseline BMI ($p = 0.053$). This could lead to underestimation of the association between BMI and bone accretion found in this study.

In conclusion, our results indicate that weight status during late adolescence could play a part in the concept of maximizing bone mass and density during growth for prevention of future fractures. ΔBW and ΔBMI predicted ΔaBMD and ΔBMC in both sexes. Although statistically significant, the magnitude of these changes in aBMD during follow-up was moderate and unlikely to have significant clinical implication on peak bone mass for adolescents with an adequate BW. Loss of BW or reduction of BMI was not associated with net loss of aBMD, but individuals who lost weight during follow-up, demonstrated a slowed progression of aBMD accretion compared with those gaining weight, especially among boys. Considering that more than one of five adolescents was classified as overweight or obese at baseline and with an increasing prevalence during follow-up for girls and boys, the bone health perspective must be compared with other health benefits. However, adequate weight is important for bone and our results indicate that underweight adolescent boys may benefit from a BMI increase. Particularly underweight individuals losing weight during this critical period of bone accretion could be at risk of a less than optimal peak bone mass acquisition, thus not achieving their full genetic potential for skeletal mass. Because of the short follow-up of 2 years, results must be interpreted with caution. Further analyses should also examine the effect of lifestyle factors present at baseline. Moreover, the cohort should be followed into adulthood to further explore factors that can alter the bone mass trajectory.

Disclosures

All authors state that they have no conflicts of interest.

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Supplemental table 1 *Characteristics at baseline survey Fit Futures 1 (TFF1) and follow-up survey Fit Futures 2 (TFF2) 2 years later: Continuous variables presented as mean (standard deviation) and categorical variables in percentage. The Tromsø Study, Fit Futures.*

		Girls					Boys				
		TFF1		TFF2			TFF1		TFF2		
		n		n		*p*	n		n		*p*
Age		355	16.61 (0.387)	355	18.60 (0.40)		296	16.60 (0.37)	296	18.65 (0.35)	
Age groups at baseline:	15	9	2.5 %				19	6.4 %			
	16	294	82.8 %				238	80.4 %			
	17	52	14.7%				39	13.2 %			
Height (cm)		355	165.03 (6.48)	355	165.73 (6.57)	<.001	296	177.25 (6.52)	296	179.08 (6.49)	<.001
Weight (kg)		355	60.37 (10.61)	355	63.08 (11.94)	<.001	296	69.81 (13.68)	296	75.21 (14.64)	<.001
BMI		355	22.17 (3.76)	355	22.97 (4.18)	<.001	296	22.18 (3.93)	296	23.42 (4.18)	<.001
Sexual maturation [a]:	Early / Completed	110	31.0 %				22	9.1 %			
	Intermediate / Underway	165	46.5 %				177	72.8 %			
	Late / Barely started	73	20.5 %				44	18.1 %			
Ethnicity	White	347	97.8 %				291	98.3 %			
	Others	8	2.2 %				5	1.7 %			
Physical activity:	Sedentary	42	12.0 %	47	13.3 %		77	26.3 %	81	28.4 %	
	Moderate	141	39.5 %	144	40.8 %		75	25.6 %	60	21.1 %	
	Sports	110	30.8 %	110	31.2 %		71	24.2 %	77	27.0 %	
	Competition	63	17.6 %	52	14.7 %		70	23.9 %	67	23.5 %	
Smoking (yes)		68	19.0 %	102	28.5 %	<.001	62	20.9 %	114	38.5 %	<.001
Snuff use (yes)		108	30.2 %	152	42.5 %	<.001	108	36.5 %	142	48.0 %	<.001
Alcohol consumption (yes)		262	73.2 %	336	93.9 %	<.001	195	65.9 %	272	91.9 %	<.001
Diseases known to affect bone [b] (yes)		4	1.1 %				5	1.7 %			
Medication known to affect bone [c] (yes)		8	2.2 %				6	2.0 %			
Hormonal contraceptive use (yes)		118	33.0 %								
	Oestrogen and progestogens	105	29.3%								
	Progestogens-only	13	3.6%								
aBMD FN (g/cm^2)		355	1.07 (0.12)	355	1.07 (0.13)	0.008	296	1.11 (0.15)	296	1.14 (0.15)	<0.001
aBMD TH, (g/cm^2)		355	1.06 (0.13)	355	1.07 (0.13)	<0.001	296	1.12 (0.15)	296	1.14 (0.16)	<0.001
aBMD TB (g/cm^2)		355	1.14 (0.08)	355	1.16 (0.07)	<0.001	296	1.18 (0.10)	296	1.23 (0.09)	<0.001
BMC FN (g)		355	4.91 (0.71)	355	4.94 (0.72)	<0.001	296	5.99 (0.99)	296	6.19 (0.99)	<0.001
BMC TH (g)		355	32.01 (4.84)	355	32.42 (4.95)	<0.001	296	40.17 (6.64)	296	41.26 (6.86)	<0.001
BMC TB (g)		355	2522.89 (387.38)	355	2600.95 (381.68)	<.001	296	2963.78 (469.83)	296	3200.96 (476.10)	<0.001
BA FN (cm^2)		355	4.59 (0.34)	355	4.59 (0.34)	.866	296	5.38 (0.39)	296	5.41 (0.37)	.003

BA TH (cm^2)	355	30.15 (2.33)	355	30.20 (2.38)	.068	296	35.73 (2.47)	296	35.99 (2.51)	<.001
BA TB (cm^2)	355	2207.37 (233.59)	355	2241.68 (224.95)	<.001	296	2496.46 (240.06)	296	2598.28 (237.87)	<.001
Time between measurements (years)	355	1.95 (0.20)				296	2.01 (0.23)			

[a] Sexual maturation in girls: menarche age. Missing n=7 (1.97 %). Categories: Early (<12.5), intermediate (12.5-13.9) and late (> 14). Sexual maturation in boys: Puberty Developmental Scale. Categories: Have not begun (<2), barely started (2-2.9), underway (3-3.9) and completed (4). [b] Diseases known to affect bone (ICD10): E03 Hypothyroidism, E10 Diabetes type 1. F50.9 Eating disorders, K90.0 Celiac disease and M13 Arthritis. [c] Medication known to affect bone (ATC): D07A Plain corticosteroids, H03A Thyroid preparations, N03A Antiepileptic, R01AD Corticosteroids, R03BA Glucocorticoids (inhalants), and H02A Corticosteroids for systemic use.

Supplemental table 2 *Sensitivity analysis of crude models. Comparing regression coefficients for baseline- and changes in weight parameters during follow-up with and without baseline adjustments in femoral neck (FN) and total hip (TH) models. The Tromsø Study, Fit Futures.*

Girls

		Δ aBMD						Δ BMC					
	Baseline adjustment	FN (n=355)		TH (n=353)		TB (n=355)		FN (n=355)		TH (n=354)		TB (n=355)	
		β	p	β	*p*	β	*p*	β	*p*	β	*p*	β	*p*
Baseline body weight	unadjusted	0.003	0.134	0.002	0.191	**-0.001**	**0.246**	**0.009**	**0.355**	**0.100**	**0.089**	**-16.315**	**0.010**
	adjusted	0.003	0.099	0.003	0.116	**0.002**	**0.184**	**0.024**	**0.029**	**0.171**	**0.013**	**5.562**	**0.559**
Δ Body weight	unadjusted	0.004	0.062	0.005	0.006	0.002	0.058	**0.018**	**0.067**	0.213	<0.001	52.203	<0.001
	adjusted	0.004	0.057	0.005	0.005	0.002	0.026	**0.026**	**0.008**	0.221	<0.001	54.730	<0.001
Baseline BMI	unadjusted	0.001	0.602	0.002	0.230	**-0.002**	**0.048**	0.001	0.879	0.081	0.173	**-18.844**	**0.003**
	adjusted	0.001	0.546	0.002	0.335	**0.000**	**0.925**	0.009	0.378	0.112	0.076	**-7.260**	**0.335**
Δ BMI	unadjusted	0.001	0.538	0.004	0.035	0.001	0.200	0.013	0.177	0.175	0.003	52.960	<0.001
	adjusted	0.001	0.560	0.004	0.030	0.002	0.110	0.015	0.125	0.181	0.002	54.627	<0.001

Boys

		Δ aBMD						Δ BMC					
	Baseline adjustment	FN (n=296)		TH (n=296)		TB (n=296)		FN (n=296)		TH (n=296)		TB (n=296)	
		β	p	β	*p*	β	*p*	β	*p*	β	*p*	β	*p*
Baseline body weight	unadjusted	0.006	0.016	0.006	0.016	**0.001**	**0.688**	**0.018**	**0.368**	0.232	0.049	**3.407**	**0.706**
	adjusted	0.008	0.005	0.008	0.005	**0.006**	**0.002**	**0.059**	**0.009**	0.072	0.023	**33.515**	**0.008**
Δ Body weight	unadjusted	0.008	0.010	0.008	0.002	0.009	<0.001	0.067	0.001	0.552	<0.001	94.428	<0.001
	adjusted	0.007	0.015	0.008	0.002	0.008	<0.001	0.064	0.001	0.548	<0.001	93.669	<0.001
Baseline BMI	unadjusted	0.003	0.309	0.005	0.049	**0.000**	**0.990**	**0.023**	**0.264**	0.174	0.140	**0.583**	**0.949**
	adjusted	0.006	0.076	0.006	0.021	**0.005**	**0.021**	**0.659**	**0.002**	0.222	0.082	**16.358**	**0.130**
Δ BMI	unadjusted	0.003	0.290	0.005	0.079	0.006	0.001	0.033	0.106	0.350	0.004	78.477	<0.001
	adjusted	0.003	0.333	0.004	0.083	0.006	0.001	0.031	0.120	0.347	0.005	77.863	<0.001

aBMD =Areal bone mineral density (g/cm2), BMC = Bone mineral content (g), FN = Femoral neck, TH = Total hip, BMI= Body mass index (kg/cm^2), body weight in kg, Δ= change. All β coefficients are per SD change in exposure. All coefficients are per SD change in exposure. Disagreement between models in bold

Paper III

Nilsen OA, Emaus N, Christoffersen T, Winther A, Evensen E, Thrane G, Furberg AS, Grimnes G, Ahmed LA. The influence of snuff and smoking on bone accretion in late adolescence. The Tromsø Study, Fit Futures. *Submitted.*

The influence of snuff and smoking on bone accretion in late adolescence. The Tromsø study, Fit Futures

Authors / Affiliations:

Ole Andreas Nilsen, Department of Health and Care Sciences, UiT The Arctic University of Norway.

Nina Emaus, Department of Health and Care Sciences, UiT The Arctic University of Norway.

Tore Christoffersen, Finnmark Hospital TrustAlta, Norway.

Anne Winther, Division of Neurosciences, Orthopedics and Rehabilitation Services, University Hospital of North Norway.

Elin Evensen, Department of Clinical Research, University Hospital of North Norway.

Gyrd Thrane, Department of Health and Care Sciences, UiT The Arctic University of Norway

Anne-Sofie Furberg, Molde University College, Norway, and Department of Microbiology and Infection Control, University Hospital of North Norway.

Guri Grimnes, Division of Internal Medicine, University Hospital of North Norway and Endocrine Research Group, Department of Clinical Medicine, The Arctic University of Norway.

Luai Awad Ahmed, Institute of Public Health, College of Medicine and Health Sciences, United Arab Emirates University, Al Ain, United Arab Emirates.

Corresponding author: O.A. Nilsen Department of Health and Care Sciences, UiT The Arctic University of Norway. E-mail: ole-andreas.nilsen@uit.no. +4777625119.

Conflict of interest: Ole Andreas Nilsen, Nina Emaus, Tore Christoffersen, Anne Winther, Elin Evensen, Gyrd Thrane, Anne-Sofie Furberg, Guri Grimnes, and Luai Awad Ahmed declare that they have no conflict of interest.

Abstract

Bone mineral accrual in childhood and adolescence is a long term primary preventive strategy of osteoporosis. Areal bone mineral density (aBMD) is a surrogate measure of bone strength and a predictor of fracture risk. The aim of this population-based 2-year follow-up cohort study was to explore associations between use of snuff and smoking and changes (Δ) in aBMD in Norwegian girls and boys aged 15-17 years at baseline. The first wave of the Tromsø study, Fit Futures was conducted from 2010 to 2011. Femoral neck (FN), total hip (TH), and total body (TB) bone mineral content (BMC) and aBMD were measured by dual-energy X-ray absorptiometry. Information on use of snuff, smoking habits and other lifestyle related variables were collected through self-administered questionnaires. Two years later, during 2012-2013, the measurements were repeated in the second wave. The present study included 349 girls and 281 boys and compared "non-users" with "users" of snuff and "non-smokers" with "smokers" using linear regression. The influence of "double-use" on bone accretion was also explored.

In girls, no associations between use of snuff and ΔaBMD were found. In boys, use of snuff was associated with reduced bone accretion in all ΔaBMD models. Sensitivity analysis with exclusion of "sometimes" users of snuff strengthened associations at femoral sites in girls and attenuated all associations in boys.

In girls, no associations between smoking and ΔaBMD were found. However, the FN ΔBMC model showed a significant difference between groups in the full model. In boys, only the association with TB ΔaBMD was significant in the fully adjusted models.

In girls, "double-users" analyses showed similar association to smoking. In boys, nearly all models showed statistically significant associations with a difference of ~1-2% in ΔaBMD between non-users and double-users during two years of follow-up.

Our results indicate that tobacco use in late adolescence could be detrimental to bone accretion and may be a signal of increased fracture risk in adult life.

Introduction

Osteoporosis and its clinical manifestation, fragility fractures, constitute major public health challenges worldwide and Norway has one of the highest reported hip fracture incidences in the world [1, 2]. Areal bone mineral density (aBMD) is a non-invasive way to assess bone strength and fracture risk [3]. Bone mineral levels in the elderly is a result of peak bone mass (PBM) achieved during childhood and adolescence and subsequent age-related bone loss [4]. The bone-building years until the age of 18 are critical, as accrual of approximately 95 % of adult bone mass occurs during these years [5]. Optimizing the genetic potential of PBM in adolescence is a long term primary preventive strategy of osteoporosis. Around 20-40 % of PBM achievement is attributed to lifestyle choices and several modifiable behavioral determinants such as physical activity, body composition and use of recreational drugs have been identified [6]. Tobacco use has been associated with lower aBMD during bone-building years [7, 8].

Over the past decades, there has been an increase in the prevalence of snuff users in Norway [9]. Snuff (Swedish snus) is smokeless, oral tobacco traditionally produced and mainly consumed in the Nordic countries. Within the EU/EEA area, Norway and Sweden are currently the only two countries allowing snuff for sale. However, even though the sale of snuff is prohibited in Finland and Denmark, data collected from these countries, shows that snuff is also used there [10].

The increase in use of snuff in Norway started around the year 1990 among young men and after 2005 among women as well [11]. Whereas the amount of traditional smoking has decreased, snuff is now the most commonly used tobacco in the age range 16-24 years of age. The prevalence of daily smokers in these age groups has dropped from around 30 % to roughly 5 % in the last 20 years, while recent studies estimate that 25 % of men and 15 % of women use snuff daily [12, 13]. The prevalence of daily use of snuff is still increasing, although the rapid growth in proportion has slowed during the recent years [9].

There are constituents of snuff with a potentially wide range of adverse health effects, but these issues are relatively unexplored and evidence is controversial [14]. However, snuff and smoke tobacco expose individuals to many of the same substances, and the adverse influence of smoking on bone health in the adult population is well established [15]. The evidence of similar effects of tobacco on achievement of PBM during growth is suggestive, however, not

compelling [6-8, 16-19]. Winther and colleagues found a negative association between smoking and aBMD in boys, but no relationship between use of snuff and bone mass among Norwegian adolescents 15 to 17 years of age [20]. Apart from this cross-sectional study, the associations between snuff and bone at a population level are hardly described.

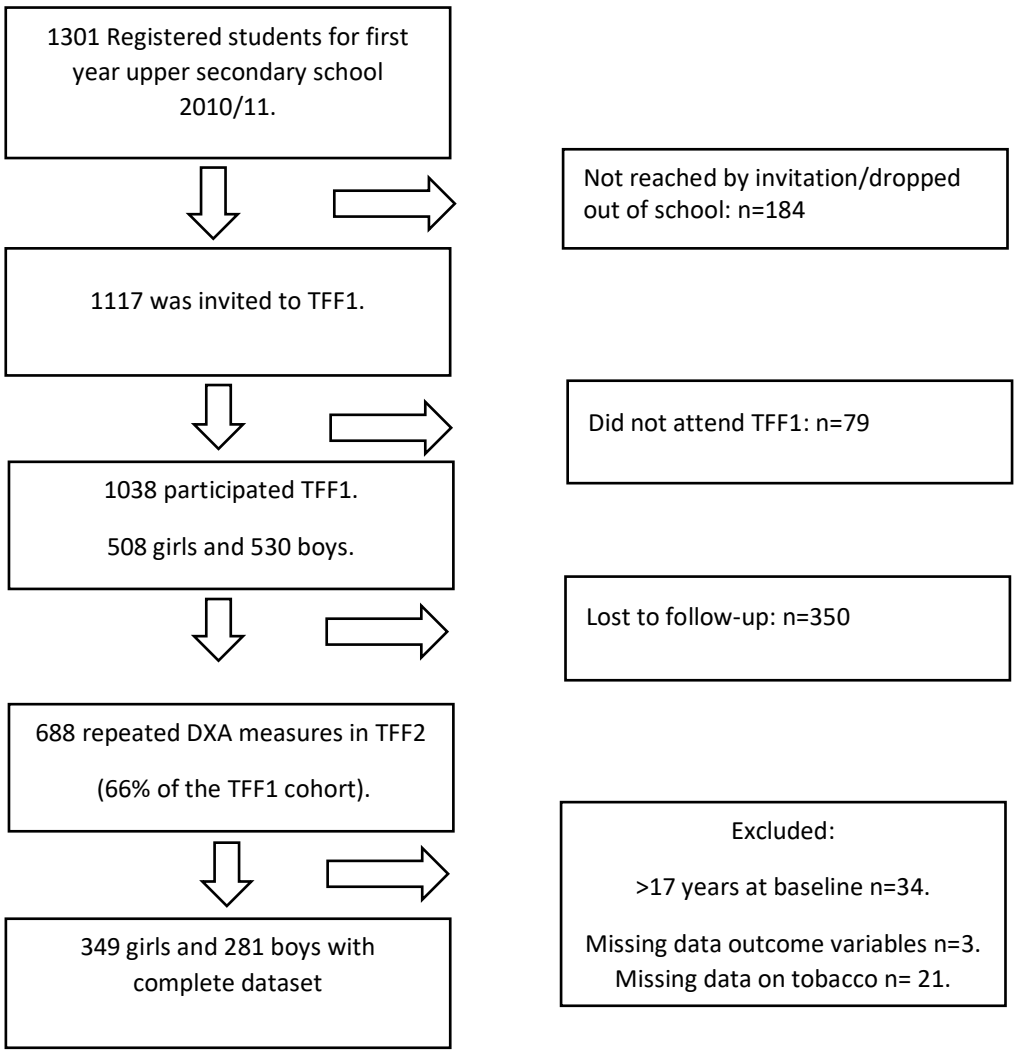
We hypothesized that both use of snuff and smoking may influence accretion of bone in adolescence. The aim of this study was therefore to explore possible association between use of snuff and change in aBMD (ΔaBMD) during a two-year follow-up in late adolescence. In addition, the associations between smoking and double use and changes in bone traits were explored.

Material and methods

Subjects

The study procedures of The Tromsø study, Fit Futures are published previously [21]. Briefly, the Fit Futures study is a school-based cohort initiated in 2010-2011 (TFF1). All first year upper-secondary school students in Tromsø and the neighbour municipalities were invited to a comprehensive health survey. Out of 1117 invited individuals, 1038 adolescents (508 girls and 530 boys) attended, giving a participation rate of 92.9 %. In the follow-up survey, Fit Futures 2 (TFF2) two years later (2012-2013), all participants in TFF1 and all new students in third year of the same upper-secondary schools were invited. A total of 66% of the TFF1 cohort met in TFF2 providing 688 repeated measures of aBMD. Participants above 17 years of age at baseline were excluded (Figure 1) and 630 individuals 15 to 17 years of age, 349 girls and 281 boys completed the questions on use of tobacco at both surveys.

Figure 1 Flowchart of participation in Fit Futures 1 (TFF1) 2010-2011 and Fit Futures 2 (TFF2) 2012-2013.



Recruitment of participants to both surveys were conducted in close collaboration with the schools. The participants received an oral briefing about the study in classrooms and were given additional written information about the study in a leaflet. The Clinical Research Unit at the University Hospital of North Norway conducted both health examinations during school days. All participants gave written informed consent at the study site. Participants younger than 16 years of age had to bring a written consent from their superiors to attend the survey. The data collection in TFF1 and TFF2 was approved by the Norwegian Data Protection Authority and the Regional Committee of Medical Research Ethics (REK nord) with a project-specific approval for the present study (Ref. 2019/31193/REK nord).

Outcome measurements

Changes in bone mineral status were measured by Dual energy X-ray Absorptiometry (DXA) as femoral neck (FN), total hip (TH) and total body (TB) bone mineral content (BMC; g) and aBMD (g/cm^2). The same instrument (GE Lunar prodigy, Lunar Corporation, Madison, WI, USA) and analytic program (Encore paediatric software [22]) were used in both TFF1 and TFF2. We used auto-analysis software and default region of interest, according to a standardized protocol. Previously, the coefficients of variation ((SD/mean] x 100) for the DXA device used have been estimated to 1.14 % at the TH and 1.72 % at the FN measured in vivo [23]. We used measurements of the left-sided hip, but in 15 cases, the data was erroneous or missing and values of the right hip was reported for both TFF1 and TFF2. The main outcome of this study was ΔaBMD, however ΔBMC is frequently reported to support the understanding of bone accretion.

Exposure variables

We collected data on use of tobacco by electronic self-administered questionnaires. At TFF1 the question “Do you use snuff?” had three alternatives: “No, never”, “Sometimes” or “Daily”. At TFF2, the answers to this question were slightly modified, and an extra alternative was added: “In the past, but not now”. If answers were “Sometimes” or “Daily”, participants were asked additional questions on frequency. The questions were: “If you use snuff sometimes, how many snuff portions do you usually take per week?” Alternatives were “One or less”, “2-3”, “4-6”, “7-10” and “More than 10”. For daily users the subsequent question was: “If you use snuff daily, how many snuff portions do you usually take per day?” Alternatives were “1”, “2-3”, “4-6”, “7-10” and “More than 10”. Information about the age of onset of use of snuff, were elicited by a question at TFF2: How old were you when you started to use snuff? The 8 alternatives were: “Below 12 years”, “12 years”, “13 years”, “14 years”, “15 years”, “16 years”, ”17 years”, ”18 years” and “19 years or above”. Questions on smoking had an identical structure as those on use of snuff at both surveys, only “portions” were replaced by “cigarettes”.

Covariates

We measured height and body weight to the nearest 0.1 cm and 0.1 kg (Jenix DS 102 Stadiometer, Dong Sahn Jenix, Korea), following standardized procedures with no shoes and light clothing. Based on these parameters, body mass index (BMI) was calculated (kg/m^2). Through clinical interviews, we assessed ethnicity, the possibility of pregnancy (exclusion criterion for DXA), acute and chronic diseases, use of medication and use of hormonal contraceptives.

Pubertal maturation information, physical activity level, and alcohol consumption were elicited by the self-administered questionnaire at TFF1. Frequency of alcohol consumption was assessed with a scale from 1 to 5: "Never", "Once per month or less", "2-4 times per month", "2-3 times per week" and "4 or more times per week". We dichotomised the answers into no (never) and yes. Covariates of pubertal maturation in boys was based on the Pubertal Developmental Scale (PDS). Secondary pubertal characteristics as growth spurt, pubic hair growth, changes in voice and facial hair growth rated on a scale from 1 (have not begun) to 4 (completed) were summarized and divided by four. We categorised a score <2 as "have not begun", 2-2.9 as "barely started", 3-3.9 as "underway" and a score of 4 as "completed" [24]. In girls, pubertal maturation was determined based on self-reported age at menarche and answers were categorised into "Early" (<12.5 years at menarche), "Intermediate" (12.5 – 13.9 years) or "Late" (> 14 years) pubertal maturation.

Self-reported physical activity level was assessed by questions from the modernized Saltin-Grimby Physical Activity Level Scale (SGPALS) [25]. The participants graded their leisure time physical activity in an average week during the last year with four alternatives: "sedentary activities only"; "moderate activity like walking, cycling or exercise at least 4 hours per week"; "participation in recreational sports at least 4 hours per week"; "participation in hard training/sports competitions several times a week". If the activity varied much, for example between summer and winter, they were asked to give an average.

Hormonal contraceptives use (girls) was categorized into "no", "estrogen and progestin" and "progestin only". We dichotomised answers on use of medication known to affect bone, and diseases known to affect bone into yes and no (medication and disease definition, see table 1).

Statistical analyses

All statistical analyses were conducted stratified by sex and characteristics of the study population are presented as means and standard deviations (SD), or count and percentages. We explored differences by ANOVA with Bonferroni correction and Pearson´s chi-squared test. We calculated absolute change (TFF2 − TFF1) and percentage change ((TFF2 − TFF1)/TFF1*100) in bone traits. Through DXA measurement dates, we were able to compute exact time of follow-up to compute annual change of anthropometric and bone parameters used in crude analyses. For simplicity purposes the snuff and cigarette frequency answers were categorized into three groups, "<1", "2-6" and ">7" units per week/day.

The hypothesis of association between the exposure of tobacco and the outcomes of ΔaBMD between TFF1 and TFF2 where investigated by linear regression models. We used TFF2 score as outcome and included the TFF1 score as a covariate to estimate the predictive value of exposure on change ($Y2 = \beta 0 + \beta 1 Y1 + \beta 2 X_{snuff} + \beta 3 \ldots$). We compared the results with change-score analysis ($Y2-Y1 = \beta 0 + \beta 1 X_{snuff}$) and explored consistency as baseline adjustments in change-score analysis may introduce bias in regression models comparing naturally occurring groups [26].

Initially we conducted crude models. Then potential confounders were added in the following way: Model 2, the "anthropometry model" comprised the crude model plus age, baseline anthropometry (height and weight) and change in anthropometric parameters. In model 3, the full model, pubertal maturation and baseline physical activity level were added to the "anthropometry model". In addition, baseline variables previously known to be of clinical importance like ethnicity, alcohol consumption, diagnosis known to affect bone, medication known to affect bone (see table 1) and hormonal contraceptives use (girls) were then added as covariates using a backwards elimination strategy where p=0.10 were used as cut-off to stay or leave the model. Based on these elimination procedures ethnicity was added to all models in boys and the TH ΔaBMD was adjusted for medication known to affect bone. In girls, hormonal contraceptives use was added to all models. All models were adjusted for time between measurements. Use of snuff models were controlled for daily smoking and vice versa.

During the first few weeks of TFF1, the questionnaire did not contain the questions related to PDS-score, giving a high percentage of missing puberty values among boys: n=52 (18.5 %).

In girls, six had missing information on menarche age. Multiple imputation based on predictors and outcome variables used in the full model were conducted to predict missing values. We assumed missing at random, 20 imputations were performed and we report pooled estimates [27]. Normal distribution, linearity, homogeneity and outliers were explored by residual analysis. In both girls and boys, one outlier was excluded in all models. In girls, the full TB BMC models residuals showed a heteroscedastic pattern and weighted least square regression were applied. We used menarche age and PDS scores as continuous variables in multiple regression models. Plausible 2-way interactions related to aBMD, age and pubertal maturation were checked and interaction terms for age*snuff were added to boys ΔaBMD TB full model, and age*double use to the ΔaBMD TB full model in girls. Significance level was set to p=0.05 and all procedures were performed in IBM SPSS Statistics for Windows, version 26 (IBM Corp., Armonk, N.Y., USA).

Results

Table 1 *Characteristics at baseline survey Fit Futures 1 (TFF1) and annual change in height, weight, areal bone mineral density and bone mineral content between TFF1 and Fit Futures 2 (TFF2) by use of snuff status: Continuous variables presented as mean (standard deviation) and categorical variables in count (percentage). The Tromsø Study, Fit Futures.*

		Girls							Boys						
		"Non-users" n=244		"Sometimes" n=51		"Daily-users" n=54		p	"Non-users" n=185		"Sometimes" n=31		"Daily-users" n=65		p
Age at baseline		16.61	(.36)	16.58	(.38)	16.67	(.48)	.483	16.61	(.35)	16.51	(.46)	16.60	(.37)	.404
Body height (cm)		165.44	(6.64)	164.21	(6.24)	164.16	(5.92)	.252	177.48	(6.59)	176.34	(6.24)	177.53	(6.66)	.651
Body weight (kg)		60.43	(10.96)	61.08	(9.39)	59.88	(10.06)	.844	68.94	(12.78)	71.11	(17.34)	71.23	(14.75)	.434
BMI, (kg/m2)		22.08	(3.80)	22.72	(3.93)	22.20	(3.45)	.546	21.85	(3.59)	22.80	(4.98)	22.56	(4.33)	.274
Maturation [a] Menarche age/PDS-score		13.02	(1.17)	12.88	(1.14)	13.02	(1.28)	.734	3.26	(.43)	3.32	(.45)	3.42	(.43)	.65
Ethnicity white/others[b]		236	(96.7%)	51	(100.0%)	54	(100.0%)	.311	183	(98.9%)	30	(96.8%)	65	(100.0%)	.414
Physical activity	Sedentary	24	(9.8%)	2	(3.9%)	15	(27.8%)		42	(22.7%)	8	(25.8%)	23	(35.4%)	
	Moderate	95	(38.9%)	18	(35.3%)	26	(48.1%)		51	(27.6%)	9	(29.0%)	12	(18.5%)	
	Sports	87	(35.7%)	16	(31.4%)	6	(11.1%)		47	(25.4%)	8	(25.8%)	14	(21.5%)	
	Competition	38	(15.6%)	15	(29.4%)	7	(13.0%)	<.001	45	(24.3%)	6	(19.4%)	16	(24.6%)	.505
Portions of snuff	<=1			28	(54.9%)	0	(0.0%)				18	(58.1%)	1	(1.5%)	
weekly/daily[c]	2-6			16	(31.4%)	27	(50.0%)				5	(16.1%)	28	(43.1%)	
	>=7			7	(13.7%)	27	(50.0%)				8	(13.7%)	36	(55.4%)	
Do you smoke?	No never	226	(92.6%)	33	(64.7%)	26	(48.1%)		177	(95.7%)	19	(61.3%)	33	(50.8%)	
	Sometimes	17	(7.0%)	15	(29.4%)	24	(44.4%)		8	(4.3%)	9	(29.0%)	27	(41.5%)	
	Daily	1	(0.4%)	3	(5.9%)	4	(7.4%)	<.001	0	(0.0%)	3	(9.7%)	5	(7.7%)	<.001
Cigarettes	<=1			43	(76.8%)	0	(0.0%)				21	(47.7%)	0	(0.0%)	
weekly/daily[c]	2-6			11	(19.6%)	6	(75.0%)				19	(43.2%)	4	(50.0%)	
	>=7			2	(3.6%)	2	(25.0%)				4	(9.1%)	4	(50.0%)	
Do you drink alcohol? (yes)		151	(61.9%)	51	(100.0%)	54	(100.0%)	<.001	92	(49.7%)	29	(93.5%)	61	(95.3%)	<.001
Diagnosis (yes)[d]		4	(1.6%)	0	(0.0%)	0	(0.0%)	1.000	4	(2.2%)	0	(0.0%)	1	(1.5%)	1.000
Medication (yes)[e]		4	(1.6%)	3	(5.9%)	1	(1.9%)	.133	6	(3.2%)	0	(0.0%)	0	(0.0%)	.345
Hormonal contraceptives	No	186	(76.2%)	28	(54.9%)	19	(35.2%)								
	Estrogen and progestin	52	(21.3%)	22	(43.1%)	29	(53.7%)								
	Progestin only	6	(2.5%)	1	(2.0%)	6	(11.1%)	<.001							
aBMD FN (g/cm^2)		1.071	(.117)	1.091	(.155)	1.051	(.122)	.252	1.108	(.150)	1.128	(.170)	1.113	(.148)	.798
aBMD TH (g/cm^2)		1.061	(.114)	1.090	(.156)	1.038	(.136)	.101	1.121	(.147)	1.143	(.171)	1.119	(.151)	.738
aBMD TB (g/cm^2)		1.142	(.074)	1.148	(.084)	1.123	(.069)	.167	1.176	(.092)	1.192	(.100)	1.194	(.101)	.349
BMC FN (g)		4.936	(.691)	4.976	(.852)	4.785	(.587)	.296	5.966	(.962)	6.121	(1.126)	6.040	(1.031)	.676
BMC TH (g)		32.137	(4.713)	32.821	(5.393)	31.025	(4.738)	.149	39.980	(6.457)	41.382	(7.546)	40.460	(6.747)	.531
BMC TB (g)		2532.361	(387.830)	2548.507	(370.368)	2481.440	(394.242)	.620	2940.101	(445.767)	2989.046	(530.478)	3021.854	(517.739)	.466
ΔBody height (cm per year)		.38	(.47)	.24	(.41)	.42	(.36)	.073	1.06	(.89)	.91	(.88)	.62	(.74)	.002
ΔBody weight (kg per year)		1.47	(2.59)	1.20	(2.43)	1.15	(2.29)	.590	3.00	(3.05)	1.99	(2.91)	2.01	(2.88)	.031
ΔaBMD FN (g/cm^2 per year)		.004	(.019)	.003	(.018)	-.004	(.019)	.019	.022	(.026)	.003	(.024)	.010	(.027)	<.001
ΔaBMD TH. (g/cm^2 per year)		.007	(.017)	.005	(.016)	-.001	(.015)	.012	.016	(.022)	.002	(.022)	.005	(.021)	<.001
ΔaBMD TB (g/cm^2 per year)		.009	(.010)	.008	(.010)	.008	(.009)	.679	.026	(.015)	.019	(.013)	.017	(.014)	<.001
ΔBMC FN (g per year)		.022	(.097)	.012	(.087)	-.013	(.091)	.047	.132	(.174)	.010	(.194)	.055	(.156)	<.001
ΔBMC TH (g per year)		.227	(.591)	.107	(.621)	.038	(.537)	.066	.772	(1.080)	.067	(.997)	.260	(.984)	<.001
ΔBMC TB (g per year)		39.680	(60.349)	47.630	(66.963)	27.898	(55.011)	.238	132.075	(81.248)	97.151	(60.081)	94.687	(69.825)	.001
Time between measurements (years)		1.925	(.198)	1.935	(.159)	2.055	(.211)	<.001	1.949	(.214)	2.078	(.218)	2.103	(.213)	<.001

Δ=change. aBMD= areal bone mineral density, BMC= bone mineral content. [a] Missing PDS score n=52. [b] Percentage of white. [c] Sometimes=weekly. [d] Diseases known to affect bone (ICD10): E03 Hypothyroidism, E10 Diabetes type 1, F50,9 Eating disorders, K90.0 Celiac disease, and M13 Arthritis. [e] Medication known to affect bone (ATC): D07A Plain corticosteroids, H03A Thyroid preparations, N03A Antiepileptic, R01AD Corticosteroids, R03BA Glucocorticoids (inhalants), and H02A Corticosteroids for systemic use. p= ANOVA.

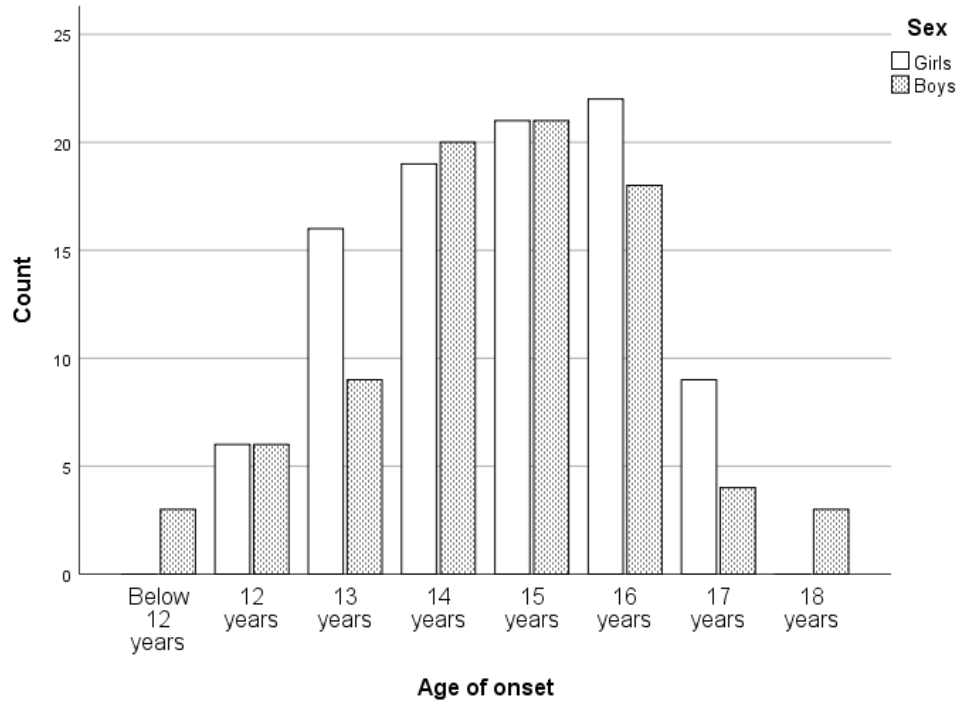
We included 630 adolescents in the present study, 349 girls and 281 boys (45 % boys) and their descriptive statistics stratified by use of snuff status are presented in Table 1. Mean age of the participants at baseline was 16.6 (SD 0.4) years with a range from 15.7 to 17.9 years. The majority were 16 years of age, 83.1 % and 80.8 % in girls and boys, respectively. At follow-up, the mean age was 18.6 (SD 0.4) years with a range from 17.8 to 20.1 years. Mean

follow-up time between TFF1 and TFF2 was 2.0 (SD 0.2) years with a range between 1.5 and 2.7 years. Drop-out analysis revealed that significantly more boys (n=196) than girls (n=111) were lost to follow-up. Girls lost to follow-up had higher BMI (0.894 kg/m^2, p=0.039), while boys had lower baseline FN aBMD (-0.028 g/cm^2, p=0.049). Both girls and boys lost to follow-up had a statistically significant higher prevalence of snuff use (girls, p=0.001; boys, p=0.005) and smoking (girls, p=0.013; boys, p=0.032).

Use of snuff

In girls, 244 (69.9 %) were classified as "non-users", 51 (14.6%) as "sometimes", and 54 (15.5 %) as "daily-users" of snuff at baseline. In boys, the corresponding numbers were 185 (65.9 %), 31 (11.0%), and 65 (23.1 %), respectively. The distribution of age at onset in the "users" group (sometimes or daily) is depicted in figure 2. The age of onset was mainly between 13 to 16 years of age and mean age was 14.6 in girls (n=93, 12 missing) and 14.7 years in boys (n=84, 12 missing; 3 participants responding onset under 12 years had age set to 11 years).

Figure 2. *Distribution of age when starting to use snuff ("users"). The Tromsø Study, Fit Futures. Girls N =93. Boys N =84.*



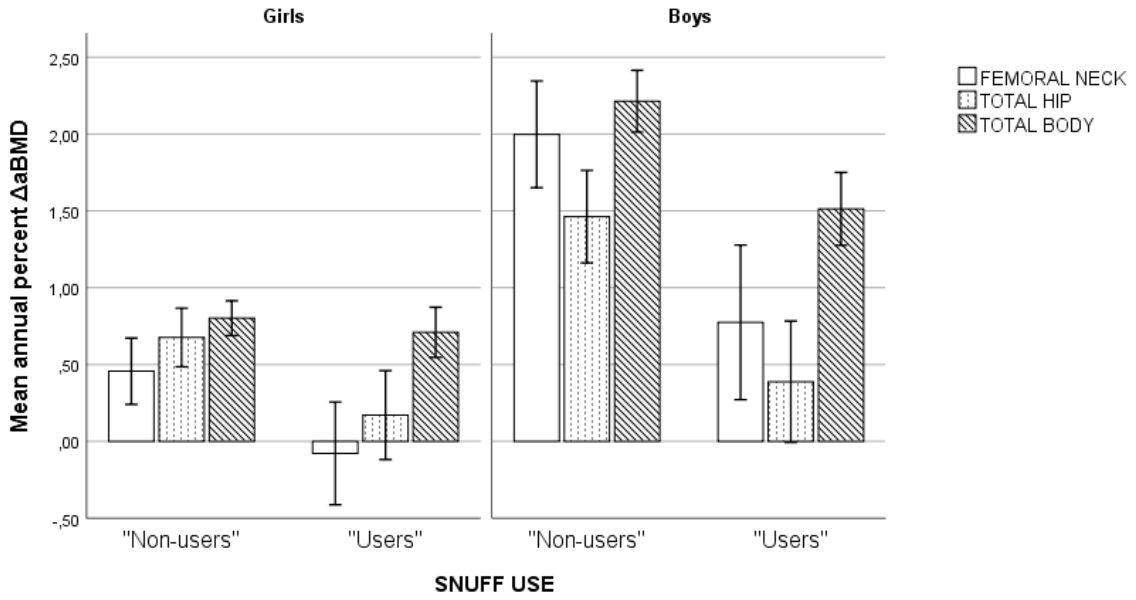
All but six of the "daily-users" at TFF1 remained "daily-users" during follow-up (51 girls and 62 boys at TFF2). The prevalence of snuff users increased during follow up. In both girls and boys, roughly 18 % "of the non-users" reported sometimes or daily use of snuff two years later. In the "sometimes" use of snuff category at baseline, more than half of the girls reported to usually take one portion or less per week and 86.3 % reported six portions per week or less. In boys, we observed corresponding numbers, with 58.1 % and 74.2 %, respectively. Furthermore, we observed that individuals in the "sometimes" category at baseline fluctuated during follow-up, and only 15 of the 82 remained in their initial "sometimes" category. In main analysis, we compared the "non-users" with the "users" of snuff, combining "sometimes" and "daily" users of snuff at baseline. Sensitivity analysis were then conducted with the "sometimes" group excluded, comparing "non-users" with "daily-users" group only.

In both girls and boys, the snuff "users" group differed significantly from the "non-users" with a higher prevalence of smokers and alcohol consumers ($p<0.001$). Among girls in the "users" category, fewer reported to be engaged in sports activities ($p<0.001$) and use of hormonal contraceptives was more prevalent in the "users" group ($p<0.001$). In boys, there was a statistically significant difference between the compared groups in annual height- ($p<0.001$), and weight change ($p=0.020$). No differences in baseline aBMD or BMC between the groups were observed.

Crude analyses of "non-users" and "users" of snuff at baseline

Crude percentage of bone accretion is shown in figure 3. In girls reporting no use of snuff at baseline, mean annual ΔaBMD (95% confidence interval) were 0.42 % (0.19, 0.64) at the FN, 0.64 % (0.43, 0.84) at the TH, and 0.79 % (0.68, 0.91) at the TB. For comparison mean annual ΔaBMD for "daily-users" were -0.08 % (-0.41, 0.26), 0.17 % (-0.12, 0.46) and 0.70 % (0.55, 0.87), respectively.

Figure 3 *Crude comparisons of "non-users" and "users" of snuff with regard to mean annual percent change in Areal bone mineral density (aBMD) between baseline and follow up measurement two years later for femoral neck, total hip and total body. The Tromsø study, Fit Futures. Girls N =349 (244/105). Boys N =281 (185/96).*



In boys, annual changes were, 2.00 % (1.65, 2.35) at FN, 1.46 % (1.16, 1.76) at the TH, and 2.21 % (2.01, 2.41) at the TB in category “non-users” of snuff. In “users” the changes were 0.77 % (0.27, 1.28), 0.38 % (-0.01, 0.78), and 1.51 % (1.28, 1.75), respectively.

Adjusted analyses of snuff use at baseline

The results of crude and adjusted regression models of ΔaBMD and ΔBMC in relation to use of snuff are presented in table 2. In girls, the “users” of snuff group had significantly less ΔaBMD compared to the “non-user” group in crude models at both femoral sites (FN: β=-.004, p=.028 and TH: β=-.019, p=.020), but not at TB. No associations were significant in the fully adjusted models.

In boys, statistically significant associations were observed in both ΔaBMD and ΔBMC, except in the adjusted ΔBMC TB models. Estimated ΔaBMD between “non-users” and “users” of snuff at the FN was 0.012 and 0.015 g/cm2 at the TH in the full models, a difference comprising roughly 1 % change during follow-up. In anthropometry models, particularly changes in anthropometric measures attenuated the associations.

Table 2 *Crude and adjusted associations between snuff use at baseline (use versus non-use) and change in femoral neck (FN), total hip (TH) and total body (TB) areal bone mineral density and bone mineral content during about 2 years follow up, adjusted for baseline measurement. The Tromsø study, Fit Futures. Girls N =348 (243/105). Boys N =280 (184/96).*

	Use of snuff	**FN**		**TH**		**TB**	
		β	*p*	*β*	*p*	*β*	*p*
	Girls n=348						
ΔaBMD	Crude	**-.004**	**.028**	**-.009**	**.020**	-.002	.418
	Age and anthropometry	-007	.084	-.007	.063	-.001	.793
	Full model	-.004	.304	-.003	.443	.000	.862
ΔBMC	Crude	**-.045**	**.036**	**-.310**	**.018**	-1.848	.893
	Age and anthropometry	-.034	.100	-.245	.052	10.107	.404
	Full model	-.073	.057	-.122	.357	-9.454 §	.783
	Boys n=280						
ΔaBMD	Crude	**-.024**	**.000**	**-.019**	**.001**	**-.015**	**.000**
	Age and anthropometry	**-.017**	**.009**	**-.013**	**.014**	**-.009**	**.010**
	Full model	**-.015**	**.023**	**-.012 #**	**.027**	**-.322 ¤**	**.019**
ΔBMC	Crude	**-.170**	**.000**	**-1.095**	**.000**	**-74.395**	**.000**
	Age and anthropometry	**-.120**	**.004**	**-.712**	**.003**	-25.073	.084
	Full model	**-.099¤**	**.020**	**-.779**	**.007**	-25.021	.098

Values are based on linear regression analysis. Δ=change. aBMD= areal bone mineral density, BMC= bone mineral content. Non-users: girls n=243 and boys n=184. Users: girls n=105 and boys n=96. Anthropometry model: crude model + age, body height, body weight, Δbody height, and Δbody weight. Full model: anthropometry model +pubertal maturation, physical activity level, daily smoking, hormonal contraceptives use (girls only) and ethnicity (boys only). All models were adjusted for time between measurements. #Adjusted for medication known to affect bone. § n=342 because of weighted least square model. ¤ Interaction age*snuff use β=.019, p=.022.

Sensitivity analysis

In analysis where the "sometimes" group was excluded, comparing "non-users" (girls, n=244; boys, n=185) with "daily-users" (girls, n=54; boys, n=65) of snuff, showed estimates with negative associations between snuff use and bone accrual. In girls, the "daily-users" of snuff group had significantly less ΔaBMD at FN compared to the "non-user" group in adjusted models: β=-.012, p=.037, while the TH association also strengthened (β=-.009, p=.071). In boys, both ΔaBMD and ΔBMC crude models were statistically significant. However, in partly adjusted models, all associations were attenuated and turned out insignificant. In the fully adjusted models use of snuff was not statistically significantly associated with ΔaBMD.

In sensitivity analysis related to multiple imputation and the high percentage of missing puberty data in boys, the full models with the original sample ("non-users vs "users" with original PDS-score, n=229) showed similar estimates, but FN; β=-.015, p=.059 and TB; β=-.008, p=.062) turned out insignificant.

In elaborate analyses of the influence of baseline snuff use and follow-up bone mineral status, we compared TFF2 aBMD and BMC parameters between the TFF1 “users” and “non-users” group to see if the two groups differed in bone traits at 17-19 years of age. Overall, “non-users” showed a mean ~1% advantage on their peers consuming tobacco at baseline, but not statistically significant (data not shown).

Association between smoking and bone accrual

There was a limited number of daily smokers at baseline in the study population, eight girls (2.3 %) and eight boys (2.8 %). In the “sometimes” category consisting of 56 girls (16 %) and 44 (15.7 %) boys, 76.8 % of the girls and 47.7 % of the boys reported to smoke one or less cigarette a week. In order to obtain a larger comparison group and to enhance exposure of smoking in the “smokers” category, the “one or less” responders were regarded as non-smokers and a combined “daily/more than 2 cigarettes weekly” category consisting of 21 girls and 31 boys was created.

No statistically significant differences in baseline bone traits between smokers and non-smokers were observed. Otherwise, the groups differed with a higher prevalence of snuff use, alcohol consumption and lower physical activity levels in the smokers group compared to the non-smokers group. Crude comparisons of change in ΔaBMD are shown in figure 4.

The results of crude and adjusted regression models of ΔaBMD and ΔBMC in relation to smoking are presented in table 3. In girls, no associations between smoking and ΔaBMD were observed, but accumulation of BMC at the FN appeared to be reduced (β=.109, p=.006).

In boys, the full ΔaBMD TB model was statistically significant (β=-.011, p=.037). The association for TH in the crude and anthropometric models was statistically significant, but was attenuated in the full model with a borderline association (p=.052).

Figure 4 *Crude comparisons of "non-smokers" and "smokers" with regard to mean annual percent ΔaBMD between baseline and follow up measurement two years later for femoral neck, total hip and total body . The Tromsø study, Fit Futures. Girls, N =349 (228/21). Boys, N = 281 (250/31).*

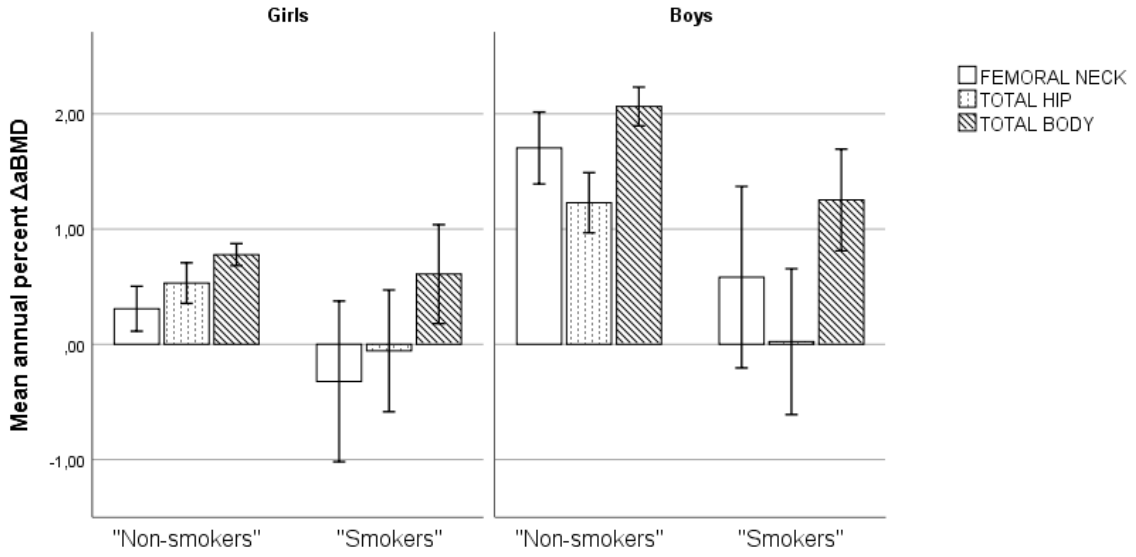


Table 3 *Crude and adjusted associations between smoking status (smoker versus non-smoker) at baseline and follow up measurement two years later for femoral neck (FN), total hip (TH) and total body (TB) aBMD and BMC, adjusted for baseline measurement. The Tromsø study, Fit Futures.*

	Smoking	FN		TH		TB	
		ß	*p*	*ß*	*p*	*ß*	*p*
	Girls n=348						
ΔaBMD	Crude	-.011	.121	-.011	.128	-.004	.322
	Age and anthropometry	-.011	.142	-.013	.063	-.004	.344
	Full model	-.009	.288	-.009	.206	-.003	.521
ΔBMC	Crude	**-.096**	**.019**	-.343	.176	.342	.990
	Age and anthropometry	**-.105**	**.008**	**-.487**	**.046**	-11.898	.613
	Full model	**-.097**	**.012**	-.348	.169	-22.099*	.264
	Boys n=280						
ΔaBMD	Crude	-.009	.398	**-.021**	**.016**	**-.016**	**.006**
	Age and anthropometry	-.011	.225	**-.013**	**.006**	**-.013**	**.008**
	Full model	-.009	.411	-.016	.052	**-.011**	**.037**
ΔBMC	Crude	**-.165**	**.014**	**-1.127**	**.004**	-49.356	.108
	Age and anthropometry	**-.125**	**.042**	**-.945**	**.007**	-32.578	.123
	Full model	-.094	.145	-.752	.050	-24.040	.293

Values are based on linear regression analysis. Δ=change. aBMD= areal bone mineral density, BMC= bone mineral content. Non-smokers: girls n=327 and boys n=249. Smokers: girls n=21 and boys n=31. Anthropometry model: crude model + age, body height, body weight, Δbody height, and Δbody weight. Full model: age and anthropometry model +pubertal maturation, physical activity level, daily snuff use and ethnicity (boys only). All models were adjusted for time between measurements.*n=342 because of weighted least square model.

Double users

At baseline, 46 girls and 44 boys responded that they were double users, indicating responses of either "Daily" or of "Sometimes", for both smoking and use of snuff at baseline. Among the "daily-users" of snuff, 28 (51.9%) of the girls and 32 (49.2%) of the boys reported to be double users, i.e. both smokers and daily use of snuff. These individuals were mostly sometimes smokers as only eight girls and eight boys reported smoking daily. Only one out of the 16 daily smokers at baseline was not double user.

The baseline differences between double users and non-users were similar to use of snuff, except that annual change in body weight did not differ in boys. The results of crude and adjusted regression models of ΔaBMD, ΔBMC and double use are presented in table 4.

In girls, no relationship was observed between ΔaBMD and double use, while the ΔBMC was significantly reduced at the FN (p=.018). In boys, most models turned out statistically significant, except the adjusted ΔaBMD FN models. An estimated difference in ΔaBMD between "non-users" and "double-users" at the TH of 0.018 g/cm^2 corresponds to ~1.6 % change during follow-up.

Table 4 *Crude and adjusted associations between double use of tobacco and change in femoral neck (FN), total hip (TH) and total body (TB) aBMD and BMC during two years follow up, adjusted for baseline measurement. The Tromsø study, Fit Futures.*

	Double-users	**FN**		**TH**		**TB**	
		ß	*p*	*ß*	*p*	*ß*	*p*
	Girls n=348						
ΔaBMD	Crude	-.009	.123	-.006	.216	-.003	.293
	Age and anthropometry	-.010	.074	-.008	.114	-.003	.263
	Full model	-.009	.114	-.004	.370	**-.249 §**	**.020**
ΔBMC	Crude	**-.066**	**.024**	-.276	.122	2.794	.881
	Age and anthropometry	**-.073**	**.009**	**-.337**	**.048**	-2.055	.900
	Full model	**-.068**	**.018**	-.210	.224	-4.514 *	.728
	Boys n=280						
ΔaBMD	Crude	**-.021**	**.017**	**-.024**	**.001**	**-.018**	**<.001**
	Age and anthropometry	-.012	.128	**-.018**	**.005**	**-.013**	**.002**
	Full model	-.012	.124	**-.018**	**.007**	**-.013**	**.002**
ΔBMC	Crude	**-.161**	**.005**	**-1.127**	**.004**	**-64.944**	**.013**
	Age and anthropometry	**-.105**	**.045**	**-.933**	**.003**	-32.578	.123
	Full model	**-.103**	**.047**	**-.898**	**.003**	-32.191	.073

Values are based on linear regression analysis. Δ=change. aBMD= areal bone mineral density, BMC= bone mineral content. Non double users: girls n=302 and boys n=236. Double-users (snuffing and/or smoking daily): girls n=46 and boys n=44. Anthropometry model: crude model + age, body height, body weight, Δbody height, and Δbody weight. Full model: anthropometry model + pubertal maturation, baseline physical activity level and ethnicity (boys only). All models were adjusted for time between measurements.*n=342 because of weighted least square model.§ Interaction age*double use β=.015, p=.021.

Discussion

To our knowledge, this is the first population-based study to explore associations between use of snuff and bone accumulation in a young population. In girls, snuff use was not associated with bone accretion in main analyses, however with an indicated inverse association at femoral sites when "non-users" and "daily-users" of snuff were compared. In boys, negative associations between use of snuff and ΔaBMD were observed at all skeletal sites. However, in contrast to girls, associations attenuated when comparing "non-user" and "daily-users" of snuff in sensitivity analyses. Smoking had limited influence on ΔaBMD, while double use was associated with a lower rate of bone accumulation at some of the skeletal sites during follow-up, especially among boys. With a few exceptions, the regression coefficients were negative for both use of snuff, smoking and double use. However, the statistical significance of the associations was not consistent and depended on skeletal site and sex.

The use of snuff in a young population is a relatively new public health issue in Scandinavia, and the health related effects of snuff are not much studied. Winther and colleagues explored the cross-sectional associations between aBMD and use of snuff in the TFF1 study population and found no statistically significant differences between users and non-users [20]. The absence of a relationship may be explained by age of onset, duration of use and temporal sequence of events. The majority of daily users of snuff reported onset to be one or two years before participation in TFF1 and the influence of snuff on bone mass may not have been established yet. The reported average debut age for use of snuff in Norway varies as one study from 2014 reported 17.7 years of age at snuff initiation in both men and women [12] and another reported age of onset to be between 15 to 17 years of age [28]. In the present study, we explored the associations of snuff exposure and changes in aBMD during two years of follow-up in a cohort where the age of onset was mainly between 13 to 16 years.

In a study from 2007, no delayed bone healing was observed in male users of snuff after osteotomy [29], but no other population-based studies on the snuff and bone relationship were found. Some studies have shown that snuff use status and periodontal bone loss are related [30], but these findings concerning the oral cavity are not necessarily comparable to skeletal

health. There are some studies showing that smokeless tobacco (chewing tobacco, non-combustible tobacco) accelerates age-related loss of aBMD in various populations, typically in India [31], Turkey [32], and in older multi-ethnic women [33, 34]. However, it has been argued that Swedish snuff has a lower potential of harm than tobacco products consumed by populations in other geographical areas worldwide [35] and thus, may not be comparable with these other types of substances related to bone.

One crucial confounder in this study is the influence of pubertal maturation. The developmental differences of normal puberty are large and hormonal status influences timing of bone accretion. The rate of bone accretion largely depends on biological rather than chronological age [5]. In girls, there was a dose-response relationship between "no never", "sometimes" and "daily" use of snuff and ΔaBMD. In boys, the "sometimes" group gained less on average at femoral sites than the "daily-users" did. This could explain why sensitivity analysis showed no differences between the "daily-users" and "non-users" of snuff. The "sometimes group" did, however, have a higher initial aBMD value. The attenuation of the associations by changes in anthropometrics in boys could indicate that some of the variation in bone accretion is due to differences in maturation not explained by pubertal maturation variable PDS score. The precision of self-reported PDS-score has been questioned [36], and use of snuff could be influenced by timing of pubertal maturation, as previously reported for smoking and alcohol consumption in another Norwegian cohort [37]. If pubertal maturation is critical to align individuals by biological age the multiple imputation process may have an impact on the results because a relatively large number of PDS scores were imputed. Sensitivity analyses without imputed data indicated this as they attenuated the associations leaving only the ΔaBMD TH model statistically significant.

The influence of smoking on PBM has been investigated more thoroughly; however, the evidence is not compelling and limited by methodological challenges. Weaver et al [6] identified 6 prospective and 7 cross-sectional studies published since year 2000 with inconsistent conclusions, but overall evidence supported the notion that smoking may have a deleterious influence on PBM. Discrepancy of associations may be due to diverse classifications of smoking status employed or frequency- and duration-dependent effects of smoking on bone. The low prevalence of regular smoking frequently limits statistical power [6]. Our study was no exception. Dorn and colleagues [7] found that the effect of smoking on bone accrual became more pronounced as girls got older. We could not confirm this relationship in the TFF cohort, but this may be related to sample size, low prevalence of

smokers and degree of exposure. Operationalization of the combined smoking category in the present study is debatable. The main concerns are whether “more than 2 cigarettes weekly” qualifies as a “smoker” and insufficient statistical power.

Adolescence is decisive for establishing habits and the high degree of tracking of smoking habits from adolescence into adulthood may possibly increase the risk of future low bone mass for these individuals [6]. Individuals that smoke (or use snuff) may be more likely than their non-smoking peers to have a lifestyle that could negatively affect aBMD and it may be hard to disentangle these factors. Essential confounders like body weight and physical activity were adjusted for in the full models. Together with changes in anthropometry, smoke was the major confounder of the relationship between bone and use of snuff and the same tendency was observed for snuff on smoke. This gave the grounds for analysis of double users.

Mechanisms

Potential pathophysiologic mechanisms of the adverse effects of tobacco on bone remains to be clarified [38]. Snuff may have different effects on bone than smoking does, because it does not undergo combustion. Nevertheless, the influence of tobacco on the skeleton may be both indirect and direct. Their common denominator is disturbance or imbalance of osteoclast and osteoblast activity [38].

One of the indirect factors may be influence of appetite and successively change in body weight, which is a determinant of aBMD [39]. In our study we found no difference in body weight or BMI in non-user and users of snuff or smokers. Tobacco could reduce the blood supply to the bones because it may have a negative impact on vascular health [14]. Another indirect mechanism is by the balance in calciotropic hormones. Abnormal PTH /vitamin D axis have been suspected to influence bone metabolism, but these potential mechanisms are mostly untested hypothesis [38] .

The direct mechanism of nicotine may have an effect on bone metabolism as it reduces osteoblast production leading to suppressed bone formation [40, 41]. Studies suggest that snuff generates the same amount of blood plasma nicotine level as smoking [42]. There is a faster uptake of nicotine by smoking, but the blood plasma nicotine level remains higher over a longer period of time by use of snuff [43]. However, the influence of nicotine on bone may be different in growing and mature skeletons [44]. Other bone-related factors affected by use of tobacco is lower cortisol- and estradiol levels and impaired collagen metabolism [45].

Study strengths and limitations

The main strengths of this study are the population-based design and a relatively large, well-described, study population where both sexes are represented. The Tromsø Study, Fit Futures is one of few studies investigating adolescent's health and lifestyle. Trained personnel at the research unit at the University Hospital of North Norway conducted the data collection in order to minimize measurement error. However, the study has some limitations to consider. Only two measurement points and the relatively short follow-up time made it challenging to differ true change in aBMD from measurement error and regression to the mean may be an issue. Even though the precision of DXA is good at a group level, the mean changes of less than one percent is debatable given the CV of the DXA scanner. DXA has limitations because of the two-dimensional measurement of BMD, leading to overestimation of larger bones. The assessment of tobacco exposure by self-administered questionnaire may induce social desirability bias and underreporting of exposure [46]. Loss to follow-up bias may influence the validity of this study, as the study sample comprises roughly 66 % of the original cohort. However, a high attendance rate at baseline (93%) contributed to the information of the drop-out analysis. Proportions of smokers and users of snuff were higher in drop-outs, which could lead to an underestimation of the associations between tobacco and bone accrual in the study. The use of baseline adjustments in two-wave observational studies with naturally occurring groups is debated. Baseline adjustments combined with measurement error may lead to directional bias leaving hypothesis tests vulnerable to type-1 error [47], and comparison with simple change scores without baseline adjustments were conducted according to advices by van Breukelen [26]. When the two approaches do not agree, results should be interpreted with caution. The disagreement was limited to 1 out of 36 models (Supplemental table). Nevertheless, disagreement may also partly be explained by the fact that one approach estimates the total effect of exposure on bone accrual, while the other estimates the direct effect, adjusting for the initial bone trait level [48].

Conclusion

Our findings suggest an inverse association between use of snuff and aBMD changes in late adolescence. In girls, no differences between "non-users" and "users" were identified, but snuff use was associated with lower femoral ΔaBMD when comparing "non-users" with "daily-users" of snuff only. In boys, negative associations between use of snuff and ΔaBMD were observed at all skeletal sites. However, in contrast to girls, associations attenuated when

comparing “non-user” and “daily-users” of snuff in sensitivity analyses. The associations between smoking and change in bone traits were limited, while double use appeared to have a detrimental influence on bone accrual in boys. The results should be interpreted with caution due to limitations of the two wave design, potentially unobserved pubertal maturation interactions, low prevalence of smokers and a short follow-up time. However, the study findings partly support our hypothesis that the use of snuff and smoking are detrimental to bone accretion and should be investigated further in cohorts with multiple waves as the consumption of snuff is rising among the adolescent population and future bone health consequences are unclear.

Supplemental table *Sensitivity analysis of crude models. Comparing regression coefficients for baseline*

Use of snuff, smoking and double use with and without baseline adjustments in femoral neck (FN) and total hip (TH) models. The Tromsø Study, Fit Futures.

Girls

		ΔaBMD						ΔBMC					
	Baseline adjustment	FN		TH		TB		FN		TH		TB	
		β	p	β	*p*	β	*p*	β	*p*	β	*p*	β	*p*
Use of snuff	Unadjusted	-0.015	0.006	-0.014	0.003	-0.003	0.505	-0.051	0.017	-0.373	0.027	-16.492	0.360
	adjusted	-0.015	0.006	-0.014	0.003	-0.003	0.311	-0.073	0.010	-0.385	0.023	-20.242	0.282
Smoking	unadjusted	-0.011	0.175	-0.011	0.131	-0.003	0.434	-0.092	0.026	-0.342	0.176	0.977	0.971
	adjusted	-0.011	0.121	-0.011	0.128	-0.004	0.322	-0.096	0.019	-0.343	0.176	0.342	0.990
Double use	unadjusted	-0.009	0.130	-0.006	0.234	-0.002	0.502	-0.058	0.047	-0.264	0.139	-7.241	0.704
	adjusted	-0.009	0.123	-0.006	0.216	-0.003	0.293	-0.066	0.024	-0.276	0.122	2.794	0.881

Boys

		ΔaBMD						ΔBMC					
	Baseline adjustment	FN		TH		TB		FN		TH		TB	
		β	p	β	*p*	β	*p*	β	*p*	β	*p*	β	*p*
Use of snuff	Unadjusted	-0.020	0.012	-0.012	0.006	-0.018	<0.001	-0.151	0.003	-0.933	0.003	-80.298	0.001
	adjusted	-0.019	0.008	-0.018	0.006	-0.016	0.000	-0.146	0.004	-1.004	0.001	-76.564	0.001
Smoking	unadjusted	**-0.020**	**0.046**	-0.022	0.008	-0.019	0.001	-0.174	0.010	-1.146	0.005	-55.659	0.068
	adjusted	**-0.009**	**0.398**	-0.021	0.016	-0.016	0.006	-0.165	0.014	-1.127	0.004	-49.356	0.108
Double use	unadjusted	-0.019	0.025	-0.023	0.001	-0.018	<0.001	-0.150	0.010	-1.209	0.001	-64.798	0.013
	adjusted	-0.021	0.017	-0.024	0.001	-0.018	<0.001	-0.161	0.005	-1.127	0.004	-64.944	0.013

aBMD =Areal bone mineral density (g/cm2), BMC = Bone mineral content (g), FN = Femoral neck, TH = Total hip, TB=Total body. Disagreement between models in bold

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Appendix A

Ethical approval 2013/1459/REK nord

Ethical approval 2019/31193/REK nord

REK
REGIONALE KOMITEER FOR MEDISINSK OG HELSEFAGLIG FORSKNINGSETIKK

Region:	Saksbehandler:	Telefon:	Vår dato:	Vår referanse:
REK nord			08.10.2013	2013/1459/REK nord
			Deres dato:	Deres referanse:
			27.08.2013	

Vår referanse må oppgis ved alle henvendelser

Nina Emaus
MH bygget

2013/1459 Ungdommers livsstil og endring i beinmasse mellom 16 -19 år

Forskningsansvarlig: Universitetet i Tromsø
Prosjektleder: Nina Emaus

Vi viser til søknad om forhåndsgodkjenning av ovennevnte forskningsprosjekt. Søknaden ble behandlet av Regional komité for medisinsk og helsefaglig forskningsetikk (REK nord) i møtet 26.09.2013. Vurderingen er gjort med hjemmel i helseforskningsloven (hfl.) § 10, jf. forskningsetikklovens § 4.

Prosjektleders prosjektomtale
In the Norwegian adult population, fracture risk is among the highest reported. The foundation of fracture risk in the elderly is laid during growth, through childhood and adolescence to the achievement of peak bone mass. Previous work has highlighted the importance of the early environment and lifestyle on this achievement. This proposed project will explore factors connected to achievement of peak bone mass: i) age and peak bone mass – tracking of bone mass levels over two years in adolescence ii) the effect of baseline weight and weight changes over two years on peak bone mass and iii) the effect of smoking, snuffing, alcohol intake and physical activity on peak bone mass achievement in adolescence. Data was collected from Fit Futures, which is an extension of the population-based Tromsø Study, from August 2010 to May 2011, and from November 2012 to June 2013. The analysis will be based on bone mineral density measurements and information from questionnaires.

Vurdering

Vurdering av om samtykket fra Fit Futures er dekkende for det som skal gjøres i studien
I samtykkeskrivet er hovedområdene det forskes på angitt slik:

"Beintetthet, Diabetes, D-vitamin, Jernmangel, Genmodifisert mat, Miljøgifter, Fysisk aktivitet og overvekt, Eksem og kviser, Infeksjoner, Smerte, Øresus, Medisinbruk, Frafall fra skole og Tannhelse. Informasjonen fra undersøkelsen vil også bli brukt til forskning om de store folkehelseproblemene generelt, slik som hjerte-karsykdommer, lungesykdommer, kreft, nedsatt fruktbarhet og smerte. Det vil også bli forsket på arbeidsførhet i skole og yrke i forhold til sykdom, helse og livsstil. En del av prosjektene vil studere samspillet mellom arv, miljø og sykdom og helse; til slike prosjekter vil det bli hentet ut genetisk arvestoff fra blodprøvene. I framtiden kan data bli brukt i forskningsprosjekter som i dag ikke er planlagt. For alle slike nye prosjekter kreves det at prosjektet er godkjent av Regional komité for medisinsk og helsefaglig forskningsetikk."

Hovedformålet i den omsøkte studien er å studere bentetthet og faktorer som påvirker denne. Komiteen anser at samtykket er dekkende for det aktuelle formålet.

Anonyme / avidentifiserte opplysninger
Under punkt 4d i søknaden står det at "All data behandles anonymisert."
Vi minner om at anonyme opplysninger er opplysninger der navn, fødselsnummer og andre personentydige kjennetegn er fjernet slik at opplysningene ikke lenger kan knyttes til en enkeltperson, jf. helseregisterloven § 2 nr. 3. Det skal heller ikke være mulig å knytte opplysningene til en enkeltperson indirekte, for eksempel gjennom at andre variabler i datasettet gjør det mulig å finne fram til en person.

Avidentifiserte helseopplysninger er helseopplysninger der navn, fødselsnummer, biometriske kjennetegn og andre personentydige kjennetegn er fjernet, slik at opplysningene ikke lenger kan knyttes til en enkeltperson, og hvor identitet bare kan tilbakeføres ved sammenstilling med de opplysningene som tidligere har blitt fjernet, jf. helseregisterloven § 2 nr. 2.

Vedtak
Med hjemmel i helseforskningsloven § 10 og forskningsetikkloven § 4 godkjennes prosjektet.

Sluttmelding og søknad om prosjektendring
Prosjektleder skal sende sluttmelding til REK nord på eget skjema senest 30.06.2017, jf. hfl. § 12. Prosjektleder skal sende søknad om prosjektendring til REK nord dersom det skal gjøres vesentlige endringer i forhold til de opplysninger som er gitt i søknaden, jf. hfl. § 11.

Klageadgang
Du kan klage på komiteens vedtak, jf. forvaltningslovens § 28 flg. Klagen sendes til REK nord. Klagefristen er tre uker fra du mottar dette brevet. Dersom vedtaket opprettholdes av REK nord, sendes klagen videre til Den nasjonale forskningsetiske komité for medisin og helsefag for endelig vurdering.

Med vennlig hilsen

May Britt Rossvoll
sekretariatsleder

Kopi til: postmottak@iho.uit.no

Region:	Saksbehandler:	Telefon:	Vår dato:	Vår referanse:
REK nord	Maren Melsbø	77620748	15.10.2019	31193
			Deres referanse:	

Nina Emaus

31193 Påvirkes ungdommer beinmasse av tobakksbruk?

Forskningsansvarlig: UiT Norges arktiske universitet

Søker: Nina Emaus

Søkers beskrivelse av formål:

Den oppnådde beinmassen - peak bone mass - rundt 20 års alder har stor betydning for fremtidig bruddrisiko. Basert på data fra ungdomsundersøkelsen Fit Futures har vi gjennom flere studier vist at beinmassen fortsatt endrer seg mellom 16 - 20 år og at flere viktige livsstilsfaktorer påvirker denne. I dette arbeidet skal vi studere hvorvidt bruk av tobakk og spesielt snus, har en negativ effekt på de unges beinmasse. I den gjennomførte Fit futures-undersøkelsen i 2010-11rapporterte 19% av jentene og 30% av guttene at de var daglige snusbrukere. Vi har derfor her et godt materiale for å studere effekten av snusbruk på ungdommers beinhelse og dermed fremtidig bruddrisiko.

REKs vurdering

Vi viser til søknad om forhåndsgodkjenning av ovennevnte forskningsprosjekt. Søknaden ble behandlet av Regional komité for medisinsk og helsefaglig forskningsetikk (REK nord) i møtet 19.09.2019. Vurderingen er gjort med hjemmel i helseforskningsloven § 10.

Formål

Av søknaden følger at formålet med prosjektet er å *«studere hvorvidt bruk av tobakk og spesielt snus, har en negativ effekt på de unges beinmasse.»*

Om prosjektet

Prosjektet er en del av en ph.d.

Den 27.08.13 søkte prosjektleder om prosjektet «Ungdommers livsstil og endring i beinmasse mellom 16-19 år» (2013/1459). Prosjektet ble godkjent med sluttdato 31.12.2016.

Av søknaden til nå omsøkte prosjekt følger at: *«Prosjektet med nummer 2013/1459, som var den opprinnelige søknaden for dette doktorgradsprosjektet er i dag meldt som avsluttet. Prosjektet har forløp som planlagt, bare med forlenget tidsplan. Da REK godkjenning var utgått har vi bedt om formell avslutning av det opprinnelige prosjektet som har produsert to artikler. Nå gjenstår siste artikkel i arbeidet og det er det vi søker om godkjenning for her.»*

Prosjektstart er i søknaden satt til 02.09.19. REK forutsetter at datainnsamling ikke igangsettes før det foreligger endelig godkjenning.

Data/materiale

Data skal hentes fra Fit Futures 1 og 2, Tromsøundersøkelsen.

Data som skal innhentes er: Kjønn, alder, høyde, vekt, fysisk aktivitet, røyking, snusbruk, alkohol, pubertetsstatus, beinmasse.

Av søknaden følger at det hentes ut ca. 50 variabler på de til sammen 1.200 deltakerne.

Deltakere

Av søknaden følger at alle deltakere i Fit Futures 1 og 2 skal inkluderes, 1.200 deltakere.

Forespørsel/informasjon/samtykkeerklæring

Deltakerne i Fit Futures 1 og 2 har avgitt samtykke. Av informasjonsbrosjyren til deltakerne fremkom at hovedområdene det forskes på er:

«Beintetthet, Diabetes, D-vitamin, Jernmangel, Genmodifisert mat, Miljøgifter, Fysisk aktivitet og overvekt, Eksem og kviser, Infeksjoner, Smerte, Øresus, Medisinbruk, Frafall fra skole og Tannhelse.

Informasjonen fra undersøkelsen vil også bli brukt til forskning om de store folkehelseproblemene generelt, slik som hjerte-karsykdommer, lungesykdommer, kreft, nedsatt fruktbarhet og smerte. Det vil også bli forsket på arbeidsførhet i skole og yrke i forhold til sykdom, helse og livsstil. En del av prosjektene vil studere samspillet mellom arv, miljø og sykdom og helse; til slike prosjekter vil det bli hentet ut genetisk arvestoff fra blodprøvene. I framtiden kan data bli brukt i forskningsprosjekter som i dag ikke er planlagt.

For alle slike nye prosjekter kreves det at prosjektet er godkjent av Regional komité for medisinsk og helsefaglig forskningsetikk.»

Hovedformålet i den omsøkte studien er å studere hvorvidt bruk av tobakk påvirker ungdommers beinmasse. REK anser at de avgitte samtykker er dekkende for det aktuelle formålet.

Oppbevaring av data

Data utleveres fra Tromsøundersøkelsen som også oppbevarer/har tilgang til koblingsnøkkel.

Vedtak

Godkjent

Med bakgrunn i ovennevnte har REK ingen innvendinger til at studien gjennomføres som beskrevet i søknad og protokoll.

REK har gjort en helhetlig forskningsetisk vurdering av alle prosjektets sider og godkjenner det med hjemmel i helseforskningsloven § 10.

Prosjektet er godkjent frem til omsøkt sluttdato 01.07.2021. Data kan oppbevares for kontrollhensyn i inntil 5 år etter prosjektslutt. Etter dette skal data slettes eller anonymiseres.

Vi gjør samtidig oppmerksom på at etter personopplysningsloven må det også foreligge et behandlingsgrunnlag etter personvernforordningen. Dette må forankres i egen institusjon.

Med vennlig hilsen

May Britt Rossvoll
sekretariatsleder

Appendix B

Pamphlet of information, The Fit Futures 1

Pamphlet of information, The Fit Futures 2

Consent of participation, The Fit Futures 1

Consent of participation, The Fit Futures 2

PERSONVERN OG SIKKERHET

Alle medarbeidere som jobber med undersøkelsen, har taushetsplikt. Opplysningene som samles inn, vil bare bli brukt til godkjente forskningsformål, som beskrevet over.

Opplysningene og prøvene vil bli behandlet uten navn og fødselsnummer eller andre direkte gjenkjennende opplysninger. En kode knytter deg til dine opplysninger og prøver. Koden oppbevares separat ved Universitetet i Tromsø, og kun noen få autoriserte personer har tilgang. Den enkelte forsker får ikke tilgang til opplysninger som gjør det mulig å identifisere enkeltpersoner. Det vil ikke være mulig å identifisere deg i resultatene av studien når disse publiseres.

I noen tilfeller kan det være aktuelt å gjøre analyser av blodprøver eller genetiske analyser ved forskningsinstitusjoner i utlandet. Hvis dette gjøres, vil våre utenlandske samarbeidspartnere ikke få opplysninger som kan knytte prøvene opp mot deg som person.

Tromsøundersøkelsen gjennomfører Fit futures i samarbeid med Universitetssykehuset Nord-Norge og Nasjonalt folkehelseinstitutt. Data som samles inn på sykehuset, overføres til Universitetet i Tromsø når datainnsamlingen er avsluttet. Ingen av opplysningene som framkommer i undersøkelsen, lagres i journalsystemet på sykehuset. Databehandlingsansvarlig er Universitetet i Tromsø. Tromsøundersøkelsen administrerer utlevering av data til forskningsprosjekter. Hvem som er ansvarlig for forskningsprosjektene, finner du her http://www.tromsoundersokelsen.no. Fit futures er godkjent av Datatilsynet og Regional komité for medisinsk og helsefaglig forskningsetikk, Nord-Norge. Deltakere er forsikret gjennom Norsk Pasientskadeerstatningsordning.

FRIVILLIG DELTAKELSE

Det er frivillig å delta i studien. Du kan når som helst og uten å oppgi noen grunn trekke ditt samtykke til å delta i undersøkelsen, og dette vil ikke få noen konsekvenser for deg. Dersom du senere ønsker å trekke deg eller har spørsmål til studien, kan du kontakte Tromsøundersøkelsen, Institutt for samfunnsmedisin, Universitetet i Tromsø, 9037 Tromsø, telefon 77644816, e-post: tromsous@uit.no.

RETT TIL INNSYN OG SLETTING AV PRØVER OG OPPLYSNINGER OM DEG

Hvis du sier ja til å delta i studien, har du rett til å få innsyn i hvilke opplysninger som er registrert om deg. Du har også rett til å få korrigert eventuelle feil i de opplysningene vi har registrert. Dersom du trekker deg fra studien, kan du kreve å få slettet innsamlede prøver og opplysninger, med mindre opplysningene allerede er inngått i analyser eller brukt i vitenskapelige publikasjoner.

ENERGI

VIL DU DELTA?

Hvis du er fylt 16 år, gir du selv ditt samtykke til å delta. Du kan da signere vedlagte skjema (hvitt ark) og ta det med til undersøkelsen. Det er også mulig å undertegne skjemaet når du kommer til Forskningsposten.

Hvis du ikke er fylt 16 år, må du be dine foreldre/foresatte om lov til å delta. Da må både du og dine foreldre/foresatte signere vedlagte skjema (hvitt ark) som du tar med deg til undersøkelsen.

ANSVARLIGE FOR GJENNOMFØRING AV FIT FUTURES UNDERSØKELSEN

Fit futures ledes av en styringsgruppe, og følgende forskere er ansvarlige for gjennomføringen:

Anne-Sofie Furberg
prosjektleder, lege, Universitetssykehuset Nord-Norge
e-post: anne-sofie.furberg@unn.no, telefon 77 75 58 24

Christopher Sivert Nielsen
psykolog, Nasjonalt folkehelseinstitutt
e-post: Christopher.Sivert.Nielsen@fhi.no, telefon 21 07 82 77

Guri Grimnes
lege, Universitetssykehuset Nord-Norge og Universitetet i Tromsø
e-post: guri.grimnes@unn.no, telefon 77 66 94 83

SPØRSMÅL?

Dersom du/dere har spørsmål om undersøkelsen, kontakt Forskningsposten UNN på telefon 77 62 69 09 eller prosjektadministrator for Fit futures på telefon 930 03 925.

www.fitfutures.no

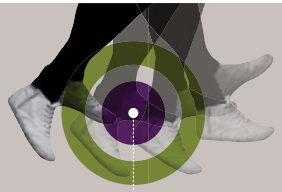
FAST FOOD

SOSIALT NETTVERK

FitFutures
EN DEL AV TROMSØUNDERSØKELSEN

DIN HELSE DIN FREMTID

INVITASJON TIL Å DELTA I HELSEUNDERSØKELSE BLANT UNGDOM



HVA ER FIT FUTURES?

Fit futures er et forskningsprosjekt der vi undersøker ungdommers fysiske helse og livsstil.

HVORFOR ER DETTE VIKTIG?

Voksnes helse undersøkes i mange studier, men man har mindre kunnskap om helse blant ungdom. Selv om få ungdommer har alvorlige sykdommer, legges mye av grunnlaget for fremtidig helse i ungdomsårene. Denne undersøkelsen kan bidra til at vi får økt kunnskap om hvordan man kan forebygge sykdom og om hvordan diagnoser kan stilles på et tidligere tidspunkt.

HVA FORSKES DET PÅ?

Hovedområdene det forskes på er:

- Eksem og kviser
- Infeksjoner
- Fysisk aktivitet og overvekt
- D-vitamin
- Jernmangel
- Genmodifisert mat
- Miljøgifter
- Smerte
- Beintetthet
- Diabetes
- Øresus
- Medisinbruk
- Frafall fra skole
- Tannhelse

Informasjonen fra undersøkelsen vil også bli brukt til forskning om de store folkehelseproblemene generelt, slik som hjerte-karsykdommer, lungesykdommer, kreft, nedsatt fruktbarhet og smerte. Det vil også bli forsket på arbeidsførhet i skole og yrke i forhold til sykdom, helse og livsstil. En del av prosjektene vil studere samspillet mellom arv, miljø og sykdom og helse; til slike prosjekter vil det bli hentet ut genetisk arvestoff fra blodprøvene. I framtiden kan data bli brukt i forskningsprosjekter som i dag ikke er planlagt. For alle slike nye prosjekter kreves det at prosjektet er godkjent av Regional komité for medisinsk og helsefaglig forskningsetikk. En oversikt over godkjente prosjekter finner du her (www.tromsoundersokelsen.no). Nettsiden holdes løpende oppdatert. Her kan du også lese om våre forskningsresultater.

HVEM KAN DELTA?

Alle ungdommer på VG1 blir invitert til å delta. Hvis du er 16 år eller mer, kan du selv bestemme om du vil delta. Er du under 16 år, må du ha samtykke fra dine foreldre eller foresatte.

AKTIVITET

SLIK FOREGÅR UNDERSØKELSEN

Undersøkelsen gjennomføres i skoletiden. Selve undersøkelsen tar 2-3 timer, og du må påregne å være borte fra skolen en halv dag. Skolen anser dette som gyldig skolefravær. Du blir undersøkt på Forskningsposten, Universitetssykehuset Nord-Norge, av erfarne forskningssykepleiere og tannleger/tannhelsesekretærer. Undersøkelsen består av følgende deler:

- *Spørreskjema* der vi spør om livsstil, trivsel, sykdommer og helseplager gjennom livet, og familieforhold.
- *Intervju* der vi spør om hvilke medisiner du bruker, om du har noen sykdom i dag og litt om sosialt nettverk. Kvinner spørres også om menstruasjon og graviditet.
- *Generell helseundersøkelse* der vi måler høyde, vekt, livvidde og hoftevidde, blodtrykk og puls, samt tar blodprøve, en hårprøve fra nakken, og en bakterieprøve fra nesebor og hals med en fuktet vattpinne.
- Måling av *smerteføl*somhet der vi måler følsomhet for trykk, kulde og varme. Smerten kommer gradvis, og du kan selv avbryte når som helst.
- *Kroppsscan* (DEXA) der vi måler beintetthet og forholdet mellom fett- og muskelvev. Dette skjer ved at du ligger rolig i ca. 10 minutter mens kroppen scannes.
- *Tannundersøkelse* som blir din årlige undersøkelse ved den offentlige tannhelsetjenesten og omfatter klinisk undersøkelse, tannrøntgen, kliniske foto og avtrykk for studiemodeller.

Etter undersøkelsen vil du få utlevert en liten *aktivitetsmåler* som er festet i et smalt strikkbelte til å ha under klærne. Denne måler hvor mye du beveger deg i løpet av dagen. Apparatet leveres på skolen etter en ukes bruk. Da vil det samtidig tas ny bakterieprøve fra nesebor og hals.

Noen deltakere vil bli forespurt om å undersøkes en gang til. Det vil da være aktuelt å gjenta noen av undersøkelsene og gjøre enkelte utvidede undersøkelser.

HVA SKJER MED DE BIOLOGISKE PRØVENE?

Med blodprøven gjøres analyser av bl.a. hormonnivåer, fettstoffer, blodsukker, vitaminer, miljøgifter og markører på betennelse og sykdommer. Det blir også hentet ut arvestoff (DNA og RNA) for genetiske analyser. Bakterieprøvene brukes til å måle forekomst av gule stafylokokker. Hårprøven analyseres for å se på nivå av kvikksølv. Prøvene lagres i Forskningsbiobanken for Tromsøundersøkelsen ved Universitetet i Tromsø. Hvis du sier ja til å delta, gir du også samtykke til at de biologiske prøvene og analyseresultatene inngår i biobanken.

INFORMASJON FRA ANDRE KILDER OG BRUK AV DATA I FRAMTIDEN

Opplysninger og prøver som du gir, blir oppbevart på ubestemt tid til bruk i forskning omkring helse og sykdom som omtalt i denne brosjyren. Det kan også hende at vi tar kontakt med deg igjen for å spørre om du vil være med på en ny undersøkelse. For spesielle forskningsprosjekter kan det være aktuelt å sammenstille informasjon fra Fit futures med nasjonale helseregistre som Reseptregisteret, Medisinsk fødselsregistrer, Kreftregisteret, Norsk pasientregister, Dødsårsaksregisteret og andre nasjonale registre over sykdommer som det forskes på i Tromsøundersøkelsen. I tillegg kan det være aktuelt å innhente helseopplysninger fra spesialist- og primærhelsetjenesten, for eksempel informasjon om beinbrudd og høyde- og vektdata fra helsestasjon, til bruk i forskning på sykdommer og helseproblemer som det forskes på i Tromsøundersøkelsen. Det kan også bli innehentet data fra registre i Statistisk sentralbyrå slik som miljø, befolkning, utdanning, inntekt, offentlige ytelser, arbeidsdeltakelse og andre forhold som kan ha betydning for helsa. For å undersøke om sykdommer går i arv, kan opplysninger om deg sammenstilles med opplysninger om dine slektninger, dersom disse har deltatt i deler av Tromsøundersøkelsen. Dette blir gjort ved å innhente opplysinger om slektskap fra Familieregisteret. Fra skolen vil vi innhente dine opplysninger om studieprogram, klasse, kjønn, antall fraværsdager, om du fullfører skoleåret og om karakterer i fagene norsk, matematikk og engelsk.

Sammenstillinger av informasjon krever noen ganger nytt samtykke og/eller annen type godkjenning slik som dispensasjon fra taushetsplikten eller godkjenning av offentlige instanser, for eksempel Regional komité for medisinsk og helsefaglig forskningsetikk, Datatilsynet eller NAV.

MULIGE ULEMPER OG FORDELER

Deltakelse innebærer at du må bruke noe tid. Deler av undersøkelsen kan også innebære ubehag. Dette gjelder særlig blodprøven. Dersom du vet at du har problemer med å ta blodprøve, kan du kontakte Forskningsposten på telefon 77 62 69 09 eller snakke med sykepleier når du kommer til undersøkelsen for å finne en løsning på dette.

Dersom resultatet av prøvene dine viser at det er nødvendig med oppfølging av tannlege, lege eller henvisning til spesialist, vil du bli orientert om det. Ved behov for henvisning til spesialist, vil vi sørge for henvisning og tilbud om oppfølging ved sykehuset.

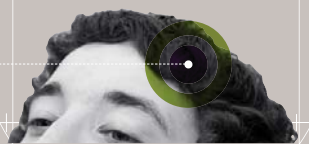
Deltakere får et gavekort til en verdi av kr. 200 ved oppmøte som kan brukes i de fleste butikker i Tromsø.

TEKNOLOGI

SMERTE

MILJØGIFTER

RØYK
OG SNUS



FitFutures
EN DEL AV TROMSØUNDERSØKELSEN

VIL DU DELTA?

Samtykke til å delta i studien Fit futures

Jeg er villig til å delta i studien

____
(DITT FULLE NAVN I BLOKKBOKSTAVER)

Sted ______________ Dato ______________

____
(DIN SIGNATUR)

- - - - - - - - - - - - - - - - - - - -

VIL DU DELTA OG ER UNDER 16 ÅR?

Foreldre/foresatte sitt samtykke til deltakelse i Fit futures

Jeg samtykker herved i at mitt/vårt barn kan delta i undersøkelsen

____
(BARNETS FULLE NAVN I BLOKKBOKSTAVER)

Sted ______________ Dato ______________

____
(SIGNATUR FORELDER/FORESATT 1)

____
(SIGNATUR FORELDER/FORESATT 2)

STØY

TANNHELSE

FRIVILLIG DELTAKELSE

Det er frivillig å delta i studien. Du kan når som helst og uten å oppgi noen grunn trekke ditt samtykke til å delta i undersøkelsen, og dette vil ikke få noen konsekvenser for deg. Dersom du senere ønsker å trekke deg eller har spørsmål til studien, kan du kontakte Tromsøundersøkelsen, Institutt for samfunnsmedisin, Det helsevitenskapelige fakultet, Universitetet i Tromsø, 9037 Tromsø, telefon 77 64 48 16, e-post: tromsous@uit.no.

HVA SKJER MED DE BIOLOGISKE PRØVENE?

Med blodprøven gjøres analyser av bl.a. hormoner, fettstoffer, blodsukker, vitaminer, miljøgifter og markører på betennelser og sykdommer. Det blir også hentet ut arvestoff (DNA og RNA) for genetiske analyser. Bakterieprøvene brukes til å måle forekomst av gule stafylokokker og meningokokker. Prøvene lagres i Forskningsbiobanken for Tromsøundersøkelsen ved Universitetet i Tromsø. Hvis du sier ja til å delta, gir du også samtykke til at de biologiske prøvene og analyseresultatene inngår i biobanken.

PERSONVERN OG SIKKERHET

Alle medarbeidere som jobber med undersøkelsen, har taushetsplikt. Opplysningene som samles inn, vil bare bli brukt til godkjente forskningsformål, som beskrevet over.

Når det forskes på data fra undersøkelsen, gjøres dette uten navn og fødselsnummer eller andre direkte gjenkjennende opplysninger. En kode knytter deg til dine opplysninger og prøver. Koden oppbevares separat ved Universitetet i Tromsø, og kun noen få autoriserte personer har tilgang. Den enkelte forsker får ikke tilgang til opplysninger som gjør det mulig å identifisere enkeltpersoner. Det vil ikke være mulig å identifisere deg i resultatene av studien når disse publiseres.

I noen tilfeller kan det være aktuelt å gjøre analyser av blodprøver eller genetiske analyser ved forskningsinstitusjoner i utlandet. Hvis dette gjøres, vil våre utenlandske samarbeidspartnere ikke få opplysninger som kan knytte prøvene opp mot deg som person.

Tromsøundersøkelsen gjennomfører Fit Futures i samarbeid med Universitetssykehuset Nord-Norge og Nasjonalt folkehelseinstitutt. Data som samles inn på sykehuset, overføres til Universitetet i Tromsø når datainnsamlingen er avsluttet. Ingen av opplysningene som framkommer i undersøkelsen, lagres i journalsystemet på sykehuset. Databehandlingsansvarlig er Universitetet i Tromsø. Tromsøundersøkelsen administrerer utlevering av data til forskningsprosjekter. Hvem som er ansvarlig for forskningsprosjektene, finner du her (www.tromsoundersokelsen.no). Fit Futures er godkjent av Datatilsynet og Regional komité for medisinsk og helsefaglig forskningsetikk, Nord-Norge. Deltakere er forsikret gjennom Norsk Pasientskadeerstatningsordning.

RETT TIL INNSYN, SLETTING AV PRØVER OG OPPLYSNINGER

Hvis du sier ja til å delta i studien, har du rett til å få innsyn i hvilke opplysninger som er registrert om deg. Du har også rett til å få korrigert eventuelle feil i de opplysningene vi har registrert. Dersom du trekker deg fra studien, kan du kreve å få slettet innsamlede prøver og opplysninger, med mindre opplysningene allerede er inkludert i analyser eller brukt i vitenskapelige publikasjoner.

BRUS & JUICE

VIL DU DELTA?

Hvis du vil delta, melder du deg på questback-link sendt til din epost eller tar kontakt med prosjektadministrator Annelene Moberg på 93 00 39 25 eller Siv Normann Gundersen på 93 00 39 54. Når du kommer til Forskningsposten på UNN, signerer du samtykkeskjema.

ANSVARLIGE FOR GJENNOMFØRING AV FIT FUTURES

Fit Futures ledes av en styringsgruppe, og følgende forskere er ansvarlige for gjennomføringen:

Anne-Sofie Furberg
prosjektleder, lege, Universitetssykehuset Nord-Norge og Universitetet i Tromsø
e-post: anne-sofie.furberg@uit.no, telefon 0 77 66

Christopher Sivert Nielsen
psykolog, Nasjonalt folkehelseinstitutt
e-post: Christopher.Sivert.Nielsen@fhi.no, telefon 21 07 82 77

Guri Grimnes
lege, Universitetssykehuset Nord-Norge
e-post: guri.grimnes@unn.no, telefon 0 77 66

Nina Emaus
professor i helsefag, Universitetet i Tromsø
e-post: nina.emaus@uit.no, telefon 77 66 07 62

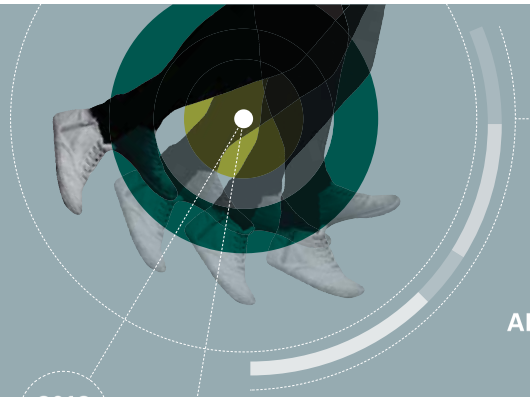
SPØRSMÅL?

Dersom du har spørsmål om undersøkelsen, kontakt:

- Prosjektadministrator **Annelene Moberg** på telefon 93 00 39 25
- Prosjektadministrator **Siv Normann Gundersen** på telefon 93 00 39 54
- **Forskningsposten UNN** på telefon 77 62 69 09

WWW.FITFUTURES.NO

UNN · Universitetet i Tromsø · folkehelseinstituttet



AKTIVITET

2012
2013

ENERGI

FitFutures 2
EN DEL AV TROMSØUNDERSØKELSEN

DIN HELSE DIN FREMTID

INVITASJON TIL Å DELTA I HELSEUNDERSØKELSE BLANT UNGDOM

MILJØGIFTER

ASTMA OG ALLERGI

HVA ER FIT FUTURES?

Fit Futures er et forskningsprosjekt der vi følger helse og livsstil fra ungdom til voksen alder. Studien begynte med undersøkelser av elever på VG 1 i Tromsø og Balsfjord skoleåret 2010-2011.

HVEM KAN DELTA?

Alle ungdommer på VG3 i Tromsø og Balsfjord blir invitert til å delta. Dette gjelder også om du er i yrkespraksis. Elever som var med i første runde av Fit Futures og siden har sluttet på skolen, er også invitert.

Vi ønsker både nye og tidligere deltakere velkommen!

HVORFOR ER DETTE VIKTIG?

Voksnes helse undersøkes i mange studier, men man har mindre kunnskap om helse blant ungdom. Selv om få ungdommer har alvorlige sykdommer, legges mye av grunnlaget for fremtidig helse i ungdomsårene. Denne undersøkelsen kan bidra til at vi får økt kunnskap om hvordan man kan forebygge sykdom og om hvordan diagnoser kan stilles på et tidligere tidspunkt. Ved å gjenta undersøkelsen kan vi følge med hvordan helsen utvikler seg over tid.

HVA FORSKES DET PÅ?

Hovedområdene det forskes på er:

- Smerte
- Eksem og kviser
- Beintetthet
- Astma og allergi
- Diabetes
- Infeksjoner
- Øresus
- Fysisk aktivitet og overvekt
- Medisinbruk
- D-vitamin
- Frafall fra skole
- Jernmangel
- Genmodifisert mat
- Miljøgifter
- Personlighet og helseatferd
- Tannhelse, syreskader og medfødte skader på tennene

Informasjonen fra undersøkelsen vil også bli brukt til forskning på de store folkehelseproblemene generelt, som hjerte-karsykdommer, lungesykdommer, kreft, nedsatt fruktbarhet og smerte. Det vil også bli forsket på arbeidsforhet i skole og yrke, knyttet til sykdom, helse og livsstil. En del av prosjektene vil studere samspillet mellom arv, miljø, sykdom og helse; til slike prosjekter vil det bli hentet ut genetisk arvestoff fra blodprøvene. I framtiden kan data bli brukt i forskningsprosjekter som i dag ikke er planlagt.

For alle slike nye prosjekter kreves det godkjenning av Regional komité for medisinsk og helsefaglig forskningsetikk. En oversikt over godkjente prosjekter finner du her: **www.tromsoundersokelsen.no**. Nettsiden holdes løpende oppdatert, og her kan du lese om våre forskningsresultater.

SLIK FOREGÅR UNDERSØKELSEN

Undersøkelsen gjennomføres i skoletiden eller arbeidstiden og tar 2–3 timer. Du må regne med å være borte fra skolen eller praksis en halv dag. Skolene anser dette som gyldig fravær. Fravær fra lærebedriften må avklares med den enkelte arbeidsgiver, men erfaringen er at de fleste arbeidsgivere gir fri for å delta i denne typen undersøkelse.

Du blir undersøkt på Forskningsposten, Universitetssykehuset Nord-Norge, av erfarne forskningssykepleiere og tannpleiere. Undersøkelsen består av følgende deler:

- *Spørreskjema* der vi spør om livsstil, trivsel, sykdommer og helseplager gjennom livet, personlighet og familieforhold.
- *Intervju* der vi spør om hvilke medisiner du bruker, om du har tatt vaksine mot smitt- som hjernehinnebetennelse, om du har noen sykdom i dag og litt om ditt sosiale nettverk. Jenter spørres også om menstruasjon og graviditet.
- *Generell helseundersøkelse* der vi måler høyde, vekt, livvidde og hoftevidde, blodtrykk og puls. Vi tar også blodprøve, spyttprøve og bakterieprøver. Bakterieprøvene tas fra nese, hals og hud med en fuktet vattpinne.
- *Kroppsscan* (DEXA) der vi måler beintetthet og forholdet mellom fett- og muskelvev. Dette skjer ved at du ligger rolig i ca. 10 minutter mens kroppen scannes.
- *Tannundersøkelse* der vi tar foto av tennene dine. Dersom du deltok i første runde av Fit Futures, vil vi også undersøke bittet ditt ved at du biter sammen med en tynn, bløt plate mellom tennene. Undersøkelsen av bittet er nødvendig for å sette sammen tannmodellene fra første runde av Fit Futures. Det vil ikke bli gjort en tannundersøkelse slik som du vanligvis får hos tannlege eller tannpleier.
- *Lungefunksjonsundersøkelse* (Spirometri) der du skal puste ut så hardt du klarer gjennom et munnstykke. Mengden luft som blåses ut, er et mål på lungefunksjonen din. Etter å ha tatt undersøkelsen en gang, vil du få puste inn en dose av astmamedisinen Ventoline® som kan utvide luftveiene dine. Deretter gjentas lungefunksjonsundersøkelsen en gang til, og vi måler om lungefunksjonen din blir bedre med medisin eller ikke.

Etter undersøkelsene vil du få utlevert en liten aktivitetsmåler som er festet i et smalt strikkbelte til å ha under klærne. Denne måler hvor mye du beveger deg i løpet av døgnet. Etter en ukes bruk, leverer du aktivitetsmåleren til prosjektadministrator på skolen.

Noen deltakere vil bli spurt om å undersøkes en gang til. Det vil da være aktuelt å gjenta noen av undersøkelsene og gjøre enkelte utvidede undersøkelser.

MULIGE ULEMPER OG FORDELER

Deltakelse innebærer at du må bruke noe tid. Deler av undersøkelsen kan også innebære ubehag, dette gjelder særlig blodprøven. Dersom du vet at du har problemer med å ta blodprøve, kan du kontakte Forskningsposten på telefon 77 62 69 09 eller snakke med sykepleier når du kommer til undersøkelsen, for å finne en løsning på dette.

Dersom resultatet av prøvene dine viser at det er nødvendig med oppfølging av tannlege, lege eller henvisning til spesialist, vil du bli orientert om det. Ved behov for henvisning til spesialist, vil vi sørge for henvisning og tilbud om oppfølging ved sykehuset.

Alle deltakere får et gavekort til en verdi av kr. 200 som kan brukes i de fleste butikker i Tromsø. Transport til og fra UNN organiseres av undersøkelsen.

TIPS OG RÅD FØR UNDERSØKELSEN

Har du fjernet halsmandlene?
Dersom du vet eller tror at du har fjernet halsmandlene, spør gjerne de hjemme om dette. Hvor gammel du var og hvorfor det skjedde. Mange får fjernet halsmandlene i småbarnsalderen, og da er det vanskelig å vite dette sikkert selv.

Bruker du astmamedisiner?
Før undersøkelsen skal du ikke bruke noen astmamedisiner som utvider luftveiene.

- Dersom du bruker Singulair, slutter du med denne 3 dager (72 timer) før undersøkelsen.
- Dersom du bruker Serevent, Oxis, Onbrez, Seretide eller Symbicort, slutter du med denne 2 dager (48 timer) før undersøkelsen.
- Dersom du bruker Ventoline, Bricanyl, Airomir, Salbutamol eller Buventol, slutter du kvelden før undersøkelsen (12 timer før).
- Det er ikke nødvendig å slutte med Pulmicort, Budesonid, Flutide, Becotide, AeroBec, Beclomet, Giona, Asmanex eller Alvesco.
- Dersom du blir verre av din astma på grunn av medisinpause, kan du likevel bruke luftveisåpnende medisiner (Ventoline, Bricanyl, Airomir, Salbutamol eller Buventol).

Bruker du andre medisiner?
Skriv ned navn på medisiner du bruker fast og ta med på undersøkelsesdagen. Har du brukt antibiotika siste 3 måneder, noter ned navnet på denne også.

INFORMASJON FRA ANDRE KILDER OG BRUK AV DATA I FRAMTIDEN

Opplysninger og prøver som du gir, blir oppbevart på ubestemt tid til bruk i forskning omkring helse og sykdom som omtalt i denne brosjyren. Det kan også hende at vi tar kontakt med deg igjen for å spørre om du vil være med på en ny undersøkelse. For spesielle forskningsprosjekter kan det være aktuelt å sammenstille informasjon fra Fit Futures med nasjonale helseregistre som Reseptregisteret, Medisinsk fødselsregister, Kreftregisteret, Norsk pasientregister, Dødsårsaksregisteret og andre nasjonale registre over sykdommer som det forskes på i Tromsøundersøkelsen. I tillegg kan det være aktuelt å innhente helseopplysninger fra spesialist- og primærhelsetjenesten og Den offentlige tannhelsetjenesten, for eksempel informasjon om beinbrudd, høyde- og vektdata fra helsestasjon, og røntgenbilder av tenner, til bruk i forskning på sykdommer og helseproblemer som det forskes på i Tromsøundersøkelsen. Det kan også bli innhentet data fra registre i Statistisk sentralbyrå slik som miljø, befolkning, utdanning, inntekt, offentlige ytelser, arbeidsdeltakelse og andre forhold som kan ha betydning for helsa. For å undersøke om sykdommer går i arv, kan opplysninger om deg sammenstilles med opplysninger om dine slektninger, dersom disse har deltatt i deler av Tromsøundersøkelsen. Dette blir gjort ved å innhente opplysninger om slektskap fra Familieregisteret. Fra skolen vil vi innhente dine opplysninger om studieprogram, klasse, kjønn, antall fraværsdager, om du fullfører skoleåret og om karakterer i fagene norsk, matematikk og engelsk.

Sammenstillinger av informasjon krever noen ganger nytt samtykke og/eller annen type godkjenning slik som dispensasjon fra taushetsplikten eller godkjenning av offentlige instanser, for eksempel Regional komité for medisinsk og helsefaglig forskningsetikk, Datatilsynet eller NAV.

FitFutures 2
EN DEL AV TROMSØUNDERSØKELSEN

VIL DU DELTA?

Samtykke til å delta i studien Fit Futures 2

Jeg er villig til å delta i studien

_____

(DITT FULLE NAVN I BLOKKBOKSTAVER)

Sted _____

Dato _____

_____

(DIN SIGNATUR)

Appendix C

Interview guide, TFF1

Printout of Electronic Questionnaire, TFF1

Extracts from the TFF2 questionnaire

Norwegian versions

Fit futures

- en del av Tromsøundersøkelsen

Intervju og Spørreskjema

Versjon: 12.04.2010

Intervju

Skriftlig samtykke:

☐ Ja ☐ Nei

Hvis nei, avbrytes undersøkelsen.

Foreldresamtykke (for de som er under 16 år)

☐ Ja ☐ Nei

Dersom de har glemt å ta med dette ber man om lov til å tas kontakt med foreldre for å innhente samtykke per telefon. To teknikere signerer på at dette er gjort.
Dersom det mangler samtykke for de under 16 år, avbrytes undersøkelsen.

Dagens dato registreres automatisk. Genererer:

[Alder i hele år]

Føler du deg frisk i dag?

☐ Ja ☐ Nei

Hvis nei:

Hva er det som feiler deg?

☐ Feber ☐ Forkjølet ☐ Hodepine ☐ Magesmerter ☐ Andre smerter
☐ Kvalme ☐ Annet
Tekstfelt for annet: _____

Har du noen form for infeksjon?

☐ Ja ☐ Nei

Hvis ja:
Beskriv: _____

Har du noen form for kroniske eller vedvarende sykdommer?
Hvor gammel var du da du fikk denne sykdommen første gang?

Diagnose 1: [ICD10 kode] Alder sykdom 1:
Diagnose 2: [ICD10 kode] Alder sykdom 2:
Diagnose 3: [ICD10 kode] Alder sykdom 3:
Diagnose 4: [ICD10 kode] Alder sykdom 4:
Diagnose 5: [ICD10 kode] Alder sykdom 5:

Tekstfelt for annet: _____

Tar du noen form for medisiner fast?

☐ Ja ☐ Nei

Hvis ja:
Medisin 1: [ATC kode]
Medisin 2: [ATC kode]
Medisin 3: [ATC kode]
Medisin 4: [ATC kode]
Medisin 5: [ATC kode]

Har du tatt noen form for smertestillende medisiner i løpet av de siste 24 timene, for eksempel Paracet, Ibux, Parlagin forte?

☐ Ja ☐ Nei

Hvis ja:

Medisin 1:	[ATC kode]	[Timer siden]	[Antall tabletter]
Medisin 2:	[ATC kode]	[Timer siden]	[Antall tabletter]
Medisin 3:	[ATC kode]	[Timer siden]	[Antall tabletter]

Har du tatt noen form for antibiotika i løpet av de siste 24 timene, for eksempel Penicillin, mot infeksjon eller kviser?

☐ Ja ☐ Nei

Hvis ja:

Medisin 1: [ATC kode]
Medisin 2: [ATC kode]
Medisin 3: [ATC kode]

Når spiste du sist?

[ ] klokkeslett – omkodes automatisk til timer siden siste måltid

Sosialt nettverkskartlegging (se redegjørelse i protokoll)

[Løpenummer venn 1]
[Løpenummer venn 2]
[Løpenummer venn 3]
[Løpenummer venn 4]
[Løpenummer venn 5]

Jenter

Har du fått menstruasjon?

☐ Ja ☐ Nei

Hvis ja (har fått menstruasjon):

Hvor regelmessig er menstruasjonene dine?

☐ Alltid regelmessig ☐ Oftest regelmessig ☐ Uregelmessig

Hvor mange dager er det mellom start av hver menstruasjon?

[Antall dager]

Hvilken dag startet siste menstruasjon? ***Dato registreres, genererer:***

[Dager siden siste menstruasjon]

Bruker du noen form for hormonell prevensjon, for eksempel p-piller?

(følges eventuelt opp med spørsmål om type prevensjon om dette ikke sies spontant)

☐ Nei ☐ P-piller ☐ P-sprøyte ☐ Annet

Er det noen mulighet for at du kan være gravid nå?
□ Ja □ Nei

Hvis ja:
Er det greit for deg at vi tar en gravitest?
□ Ja □ Nei
(resultat av prøven formidles ikke til foreldre)

Hvis ja:
Resultat av gravitest:
□ Negativ □ Positiv □ Ikke utført

Klarert for DEXA (genereres automatisk)
□ Ja □ Nei

Følgende personer er ikke klarert:
Kvinner som sier det er mulighet for at de er gravide som ikke vil gjøre gravitest
Kvinner som har positiv gravitest.

Alle: ved innsamling av aktigraf

Hvor mange timer totalt var du utendørs i dagslys i løpet av de siste 7 dagene?
[ ][ ] timer

FF - Generelt spørreskjema - Uke 1

Vi ønsker å vite mer om livsstil og helse.

Bruk den tiden du trenger til å svare så presist du kan.

Alle svarene dine blir behandlet med taushetsplikt.

Bruk "neste >>" og "<< tilbake" - knappene i skjema for å bla deg fremover og bakover.

Lykke til og tusen takk for hjelpen!

DEG OG DIN FAMILIE

1) Er du:

- ○ Jente
- ○ Gutt

2) Hvem bor du sammen med nå? (sett ett eller flere kryss)

- [ ] Mor
- [ ] Far
- [ ] 1-2 søsken
- [ ] 3 eller flere søsken
- [ ] Mors nye mann/samboer
- [ ] Fars nye kone/samboer
- [ ] Fosterforeldre
- [ ] Adoptivforeldre
- [ ] Besteforeldre
- [ ] Venner
- [ ] Alene/på hybel
- [ ] Institusjon
- [ ] Annet

3) Hvor lenge er det siden du flyttet hjemmefra?

- ( ) Mindre enn 6 måneder
- ( ) 6 - 11 måneder
- ( ) 1 - 2 år
- ( ) Mer enn 2 år

4) Er moren din i arbeid? (sett ett eller flere kryss)

- [ ] Ja, heltid
- [ ] Ja, deltid
- [ ] Arbeidsledig
- [ ] Uførerygdet
- [ ] Hjemmeværende
- [ ] Går på skole, kurs, e.l.
- [ ] Pensjonist
- [ ] Mor er død
- [ ] Vet ikke
- [ ] Annet

5) Er faren din i arbeid? (sett ett eller flere kryss)

- [ ] Ja, heltid
- [ ] Ja, deltid
- [ ] Arbeidsledig
- [ ] Uførerygdet
- [ ] Hjemmeværende
- [ ] Går på skole, kurs, e.l.
- [ ] Pensjonist
- [ ] Far er død
- [ ] Vet ikke
- [ ] Annet

6) Hva er den høyeste fullførte utdanningen til dine foreldre? (sett kryss for alle utdanningene du vet om for mor og far)

	Grunnskole	Yrkesfaglig videregående, yrkesskole	Allmennfaglig videregående skole eller gymnas	Høyskole eller universitet, mindre enn 4 år	Høyskole eller universitet, 4 år eller mer	Vet ikke
Mors utdanning	○	○	○	○	○	○
Fars utdanning	○	○	○	○	○	○

7) Hva regner du deg selv som: (kryss av for ett eller flere alternativ)

- ☐ Norsk
- ☐ Samisk
- ☐ Kvensk/Finsk
- ☐ Annet, spesifiser her

8) I hvilken kommune bodde du da du var 5-6 år (førskolealder/1.klasse)?

Velg kommune ▼

9) Er du født i Norge?

- ○ Ja
- ○ Nei, spesifiser hvilket land

10) Er din biologiske mor født i Norge?

- ○ Ja
- ○ Nei, spesifiser hvilket land

11) Er din biologiske far født i Norge?

- ○ Ja
- ○ Nei, spesifiser hvilket land

12) Har du noen gang oppholdt deg 4 uker eller mer sammenhengende i Australia, USA, Argentina eller Sør-Afrika?

- Ja
- Nei

Hvis det har vært flere opphold, oppgi varighet av siste opphold.

13) Hvor lenge varte oppholdet?

- Mindre enn 2 måneder
- 2-6 måneder
- Mer enn 6 måneder

Hvis det har vært flere opphold, oppgi når du hadde siste opphold.

14) Når var oppholdet? (Oppgi årstall når oppholdet sluttet - 4 siffer)

VENNER OG SKOLE

15) Har du vurdert å avbryte eller ta pause fra den videregående opplæringen du er i gang med?

- Ja
- Nei

16) Hvor sannsynlig er det at du fullfører den utdanningen du er i gang med?

- Liten - kommer til å slutte
- God - kommer sannsynligvis til å fullføre
- Stor - Kommer helt sikkert til å fullføre
- Vet ikke

17) Hvor mange tekstmeldinger (SMS/MMS) sendte du med mobiltelefon i går?

- Ingen
- 1-5 meldinger
- 6-10 meldinger
- 11-20 meldinger
- 21-50 meldinger
- Mer enn 50 meldinger

18) Nedenfor er det noen spørsmål om hvordan du synes du selv er. Kryss av for det som passer best for deg.

	Stemmer svært godt	Stemmer nokså godt	Stemmer nokså dårlig	Stemmer svært dårlig
Jeg synes det er ganske vanskelig å få venner	○	○	○	○
Jeg har mange venner	○	○	○	○
Andre ungdommer har vanskelig for å like meg	○	○	○	○
Jeg er populær blant jevnaldrende	○	○	○	○
Jeg føler at jevnaldrende godtar meg	○	○	○	○

19) Hvilke avgangskarakterer fikk du fra ungdomsskolen? (sett ett kryss for hvert fag)

	1	2	3	4	5	6	Husker ikke
Norsk skriftlig	○	○	○	○	○	○	○
Matematikk	○	○	○	○	○	○	○
Engelsk	○	○	○	○	○	○	○

HELSE

20) Hvordan vurderer du din egen helse sånn i alminnelighet?

- Meget god
- God
- Verken god eller dårlig
- Dårlig
- Meget dårlig

21) Hvor ofte har du i løpet av de siste 4 ukene brukt følgende medisiner?

	Ikke brukt siste 4 uker	Sjeldnere enn hver uke	Hver uke, men ikke daglig	Daglig
Smertestillende på resept (f. eks. Paralgin forte, Pinex forte)	○	○	○	○
Smertestillende uten resept (f. eks. Paracet, Pinex, Ibux)	○	○	○	○
Sovemidler	○	○	○	○
Medisin mot depresjon	○	○	○	○
Medisiner mot ADHD	○	○	○	○
Beroligende medisiner	○	○	○	○

22) Har du diabetes?

○ Ja ○ Nei

23) Har din biologiske mor diabetes?

○ Ja ○ Nei ○ Vet ikke

24) Har din biologiske far diabetes?

○ Ja ○ Nei ○ Vet ikke

25) Bruker mor insulin? (Penn eller pumpe)

Ja Nei Vet ikke

26) Hvor gammel var mor da hun fikk diabetes?

< 20 år 20 - 40 år > 40 år

27) Bruker far insulin? (Penn eller pumpe)

Ja Nei Vet ikke

28) Hvor gammel var far da han fikk diabetes?

< 20 år 20 - 40 år > 40 år

PSYKISKE VANSKER

29) Har du gått i behandling hos psykolog, psykiater eller PP-tjenesten det siste året?

Ja Nei

30) Under finner du en liste over ulike problemer. Har du opplevd noe av dette den siste uken (til og med i dag)?

	Ikke plaget	Litt plaget	Ganske mye	Veldig mye
Plutselig frykt uten grunn	○	○	○	○
Føler deg redd eller engstelig	○	○	○	○
Matthet eller svimmelhet	○	○	○	○
Føler deg anspent eller oppjaget	○	○	○	○
Lett for å klandre deg selv	○	○	○	○
Søvnproblemer	○	○	○	○
Nedtrykt, tungsindig	○	○	○	○
Følelse av å være unyttig, lite verdt	○	○	○	○
Følelse av at alt er et slit	○	○	○	○
Følelse av håpløshet med hensyn til framtida	○	○	○	○

31) De følgende spørsmålene handler om hva du følte og gjorde de siste to ukene.

	Riktig	Noen ganger riktig	Ikke riktig
Jeg var lei meg eller ulykkelig	○	○	○
Jeg følte meg så trøtt at jeg bare ble sittende uten å gjøre noen ting	○	○	○
Jeg var veldig rastløs	○	○	○
Jeg var ikke glad for noe	○	○	○
Jeg følte meg lite verdt	○	○	○
Jeg gråt mye	○	○	○
Jeg hatet meg selv	○	○	○
Jeg tenkte at jeg aldri kunne bli så god som andre ungdommer	○	○	○
Jeg følte meg ensom	○	○	○
Jeg tenkte at ingen egentlig var glad i meg	○	○	○
Jeg følte meg som et dårlig menneske	○	○	○
Jeg gjorde alt galt	○	○	○
Jeg syntes det var vanskelig å tenke klart eller å konsentrere meg	○	○	○

PUBERTET

Her har vi noen spørsmål om kroppslige forandringer som skjer gjennom ungdomstiden:

32) Har du fått menstruasjon?

Ja Nei

Hvor gammel var du da du fikk menstruasjon første gang?

33) År

Velg...

34) Måneder

Velg...

35) Har du fått eller begynt å få kjønnshår?

Ja Nei

36) Har du fått eller begynt å få bryster?

Ja Nei

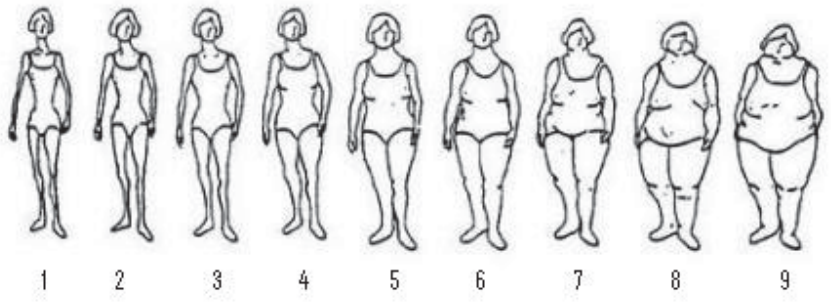
37) Har du fått eller begynt å få kjønnshår?

Ja Nei

38) Hvor gammel var du da du begynte å få kjønnshår?

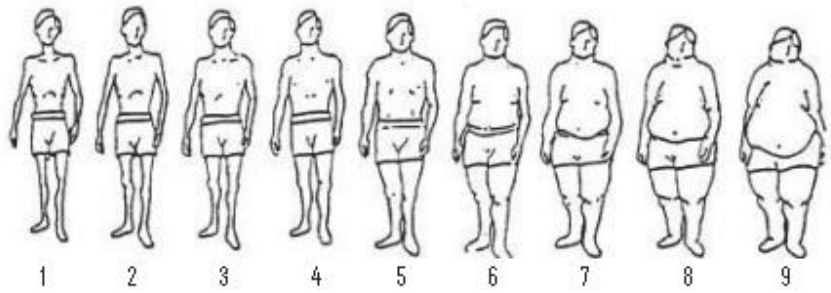
Velg...

KROPP OG VEKT



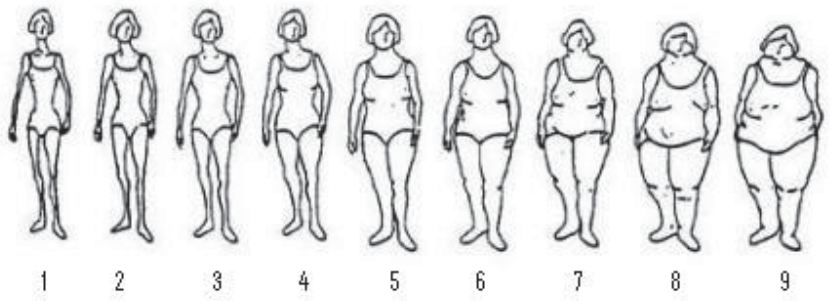
39) Hvilken av disse kroppsfasongene likner mest på kroppen til moren din?

1 2 3 4 5 6 7 8 9



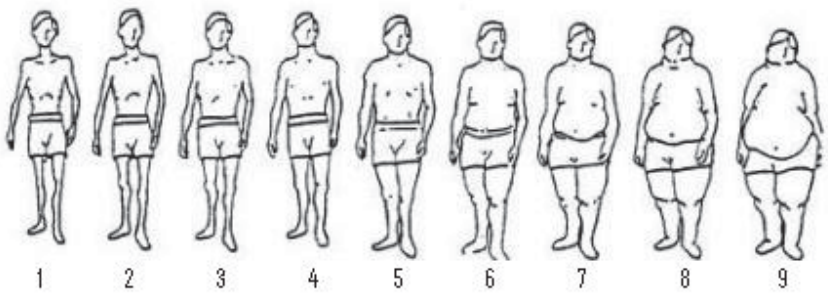
40) Hvilken av disse kroppsfasongene likner mest på kroppen til faren din?

1 2 3 4 5 6 7 8 9



41) Hvilken av disse kroppsfasongene likner mest på din kropp slik du er i dag?

○ 1 ○ 2 ○ 3 ○ 4 ○ 5 ○ 6 ○ 7 ○ 8 ○ 9



42) Hvilken av disse kroppsfasongene likner mest på din kropp slik du er i dag?

○ 1 ○ 2 ○ 3 ○ 4 ○ 5 ○ 6 ○ 7 ○ 8 ○ 9

RØYK, SNUS OG ALKOHOL

43) Røyker du?

○ Nei, aldri ○ Av og til ○ Daglig

44) Bruker du snus eller skrå?

○ Nei, aldri ○ Av og til ○ Daglig

45) Hvor mange sigaretter røyker du vanligvis i løpet av en uke?

○ 1 eller færre
○ 2-3
○ 4-6
○ 7-10
○ Mer enn 10

46) Hvor mange sigaretter røyker du vanligvis per dag?

- 1
- 2-3
- 4-6
- 7-10
- Mer enn 10

47) Hvor mange priser snus/skrå bruker du vanligvis i løpet av en uke?

- 1 eller færre
- 2-3
- 4-6
- 7-10
- Mer enn 10

48) Hvor mange priser snus/skrå bruker du per dag?

- 1
- 2-3
- 4-6
- 7-10
- Mer enn 10

49) Hvor ofte drikker du alkohol?

- Aldri
- 1 gang per måned eller sjeldnere
- 2-4 ganger per måned
- 2-3 ganger per uke
- 4 eller flere ganger per uke

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

- 1-2
- 3-4
- 5-6
- 7-9
- 10 eller flere

51) Hvor ofte drikker du 6 eller flere enheter alkohol ved en anledning?

- Aldri
- Sjeldnere enn 1 gang per måned
- 1 gang per måned
- 1 gang per uke
- Daglig eller nesten daglig

FYSISK AKTIVITET

52) Hvilken beskrivelse passer best når det gjelder din fysiske aktivitet på fritiden det siste året?

- Sitter ved PC/TV, leser eller annen stillesittende aktivitet.
- Går, sykler eller beveger deg på annen måte minst 4 timer i uken (her skal du også regne med tur til/fra skolen, shopping, søndagsturer med mer).
- Driver med idrett/trening, tyngre utearbeid, snømåking eller liknende minst 4 timer i uka.
- Trener hardt eller driver konkurranseidrett regelmessig og flere ganger i uka.

53) Hvordan kommer du deg vanligvis til og fra skolen i sommerhalvåret?

- Med bil, motorsykkel/moped
- Med buss
- Med sykkel
- Går

54) Hvor lang tid bruker du vanligvis til og fra skolen (en vei) i sommerhalvåret?

- Mindre enn 5 minutter
- 6 til 15 minutter
- 16 til 30 minutter
- 1/2 til 1 time
- Mer enn 1 time

55) Hvordan kommer du deg vanligvis til og fra skolen i vinterhalvåret?

- Med bil, motorsykkel/moped
- Med buss
- Med sykkel
- Går

56) Hvor lang tid bruker du vanligvis til og fra skolen (en vei) i vinterhalvåret?

- Mindre enn 5 minutter
- 6 til 15 minutter
- 16 til 30 minutter
- 1/2 til 1 time
- Mer enn 1 time

57) Driver du med idrett eller fysisk aktivitet (f.eks. skateboard, fotball, dans, løping) utenom skoletid?

- Ja
- Nei

58) Hvor mange dager i uken driver du med idrett/fysisk aktivitet utenom skoletid?

- Aldri
- Sjeldnere enn 1 dag i uka
- 1 dag i uka
- 2-3 dager i uka
- 4-6 dager i uka
- Omtrent hver dag

59) Omtrent hvor mange timer per uke bruker du til sammen på idrett/fysisk aktivitet utenom skoletid?

- Ingen
- Omtrent 1/2 time
- Omtrent 1 - 1 1/2 time
- Omtrent 2 - 3 timer
- Omtrent 4 - 6 timer
- 7 timer eller mer

60) Hvor slitsom er vanligvis idretten/aktiviteten du driver med utenom skoletid?

- Ikke anstrengende
- Litt anstrengende
- Ganske anstrengende
- Meget anstrengende
- Svært anstrengende

Utenom skoletid: Hvor mange timer per dag ser du på PC, TV, DVD og liknende?

61) Hverdager, antall timer per dag:

- Ingen
- Omtrent 1/2 time
- Omtrent 1 - 1 1/2 time
- Omtrent 2 - 3 timer
- Omtrent 4 - 6 timer
- Omtrent 7 - 9 timer
- 10 timer eller mer

62) Fridager (helg, helligdager, ferie), antall timer per dag:

- Ingen
- Omtrent 1/2 time
- Omtrent 1 - 1 1/2 time
- Omtrent 2 - 3 timer
- Omtrent 4 - 6 timer
- Omtrent 7 - 9 timer
- 10 timer eller mer

Svar på en skala fra 1 til 5, der 1 tilsvarer svært sjelden eller aldri og 5 tilsvarer svært ofte.

63) I hvilken grad har andre oppmuntret deg til å være fysisk aktiv

	1	2	3	4	5
Foreldre/foresatte	○	○	○	○	○
Søsken	○	○	○	○	○
Venner	○	○	○	○	○
Trenere	○	○	○	○	○
Gymlærere	○	○	○	○	○
Nabolaget	○	○	○	○	○

Svar på en skala fra 1 til 5, der 1 tilsvarer helt enig og 5 tilsvarer helt uenig.

64) Hvordan passer disse utsagnene for deg?

	1	2	3	4	5
Det er morsommere å drive med trening eller fysisk aktivitet enn å gjøre andre ting...	○	○	○	○	○
Jeg skulle ønske jeg kunne drive mer med trening eller fysisk aktivitet enn det jeg har anledning til å gjøre...	○	○	○	○	○
Jeg føler at jeg er bedre enn de fleste på min alder i idrett/fysisk aktivitet...	○	○	○	○	○
Jeg føler at jeg lett kan holde følge med de andre på min alder når vi driver med idrett/fysisk aktivitet...	○	○	○	○	○

Svar på en skala fra 1 til 5, der 1 tilsvarer helt enig og 5 tilsvarer helt uenig.

65) Hvordan passer disse utsagnene for deg?

	1	2	3	4	5
Jeg liker ikke å trene mens noen står å ser på...	○	○	○	○	○
Tilgang til egen garderobe hadde gjort det lettere å trene...	○	○	○	○	○
Jeg blir ubehagelig andpusten, svett eller får vondt i kroppen ved trening...	○	○	○	○	○
Gymtimene er organisert slik at jeg ikke henger med...	○	○	○	○	○
Jeg har ingen å trene sammen med...	○	○	○	○	○
Jeg mangler utstyr for å drive med den aktiviteten jeg har lyst til...	○	○	○	○	○
Jeg har for mange andre oppgaver som gjør at jeg ikke får tid til å trene (f.eks lekser, hjemmeoppgaver)...	○	○	○	○	○
Det mangler egnede haller eller gode uteområder for å drive fysisk aktivitet der jeg bor...	○	○	○	○	○

MATVANER OG KOSTHOLD

66) Hvor ofte pleier du å spise følgende i løpet av en uke?

	Hver dag	4-6 dager i uka	1-3 dager i uka	Sjelden eller aldri
Frokost	○	○	○	○
Middag	○	○	○	○

67) Hvor ofte spiser du matpakke hjemmefra på skolen?

- ○ Hver dag
- ○ 3-4 ganger per uke
- ○ 1-2 ganger per uke
- ○ Sjelden eller aldri

68) Hvor ofte spiser du vanligvis disse matvarene?

	Sjelden/ aldri	1-3 ganger per måned	1-3 ganger per uke	4-6 ganger per uke	Hver dag
Ost (alle typer)	○	○	○	○	○
Fet fisk (f.eks. laks, ørret, makrell, sild)	○	○	○	○	○
Mager fisk (f.eks. torsk, sei, hyse)	○	○	○	○	○
Pizza, hamburger eller pølser	○	○	○	○	○
Hermetisert mat (fra metallbokser)	○	○	○	○	○
Godteri (f.eks. sjokolade, drops)	○	○	○	○	○
Snacks og søtsaker (f.eks. potetgull, kake, kjeks, bolle)	○	○	○	○	○
Sukkerfri tyggegummi	○	○	○	○	○

69) Hvor ofte spiser du vanligvis

	Sjelden/ aldri	1-3 ganger per mnd	1-3 ganger per uke	4-6 ganger per uke	1-2 ganger per dag	3-4 ganger per dag	5 eller flere ganger per dag
Frukt	○	○	○	○	○	○	○
Grønnsaker	○	○	○	○	○	○	○

70) Hvor mange ganger i året spiser du vanligvis disse matvarene?

	0	1-3	4-5	6-9	10 eller flere
Mølje med fiskelever	○	○	○	○	○
Måsegg	○	○	○	○	○
Reinsdyrkjøtt	○	○	○	○	○
Selvplukket sopp	○	○	○	○	○

71) Hvor mye drikker du vanligvis av følgende?

	Sjelden/ aldri	1-6 glass per uke	1 glass per dag	2-3 glass per dag	4 glass eller mer per dag
Helmelk, kefir, yoghurt	○	○	○	○	○
Lettmelk, cultura, lettyoghurt	○	○	○	○	○
Skummet melk (sur/søt)	○	○	○	○	○
Ekstra lett melk	○	○	○	○	○
Juice	○	○	○	○	○
Saft med sukker	○	○	○	○	○
Lettsaft, kunstig søtet	○	○	○	○	○
Brus med sukker (1/2 liters flaske = 2 glass)	○	○	○	○	○
Lettbrus, kunstig søtet (1/2 liters flaske = 2 glass)	○	○	○	○	○
Vann	○	○	○	○	○

72) Bruker du følgende kosttilskudd?

	Ja, daglig	Iblant	Nei
Tran, trankapsler, fiskeoljekapsler	○	○	○
Vitamin- og/eller mineraltilskudd	○	○	○

SØVN OG SØVNVANER

73) Når pleier du å legge deg for å sove på ukedagene?

Velg...

74) Når pleier du å legge deg for å sove i helgen?

Velg...

75) Hvor lenge pleier du å ligge våken før du får sove på ukedagene?

Velg...

76) Hvor lenge pleier du å ligge våken før du får sove i helgen?

Velg...

77) Når pleier du å våkne på ukedagene (endelig oppvåkning)?

Velg...

78) Når pleier du å våkne i helgen (endelig oppvåkning)?

Velg...

79) Hvor mange timer sover du vanligvis pr. natt?

Velg...

80) Hvor mange timer søvn trenger du pr. natt for å føle deg uthvilt?

Velg...

81) Synes du at du får tilstrekkelig med søvn?

- Ja, absolutt tilstrekkelig
- Ja, stort sett tilstrekkelig
- Nei, noe utilstrekkelig
- Nei, klart utilstrekkelig
- Nei, langt fra tilstrekkelig

HUD

Her har vi noen spørsmål om vanlige hudplager/hudsykdommer.

82) Har du hatt kløende utslett i løpet av de siste 12 månedene?

Ja Nei Vet ikke

83) Har dette utslettet sittet på noen av de følgende stedene: rundt hals, ører eller øyne, i albuebøyene (på innsiden), under baken, bak knærne eller foran på anklene?

Ja Nei

84) Hvor gammel var du første gang du fikk denne typen utslett?

Velg...

Hvor mye plaget er du av dette utslettet i dag?
Svar på en skala fra 0-10, der 0 tilsvarer ingen plager og 10 tilsvarer verst tenkelige plager.

0 1 2 3 4 5 6 7 8 9 10

86) Har du hatt håndeksem flere ganger?

Ja Nei Vet ikke

Hvor mye plaget er du av håndeksem i dag?
Svar på en skala fra 0-10, der 0 tilsvarer ingen plager og 10 tilsvarer verst tenkelige plager.

0 1 2 3 4 5 6 7 8 9 10

88) Har du noen gang vært plaget av kviser?

Ja Nei Vet ikke

Hvor mye plaget er du av kviser i dag?
Svar på en skala fra 0-10, der 0 tilsvarer ingen plager og 10 tilsvarer verst tenkelige plager.

0 1 2 3 4 5 6 7 8 9 10

90) Har du noen gang oppsøkt lege på grunn av kviser?

Ja Nei

91) Har du fått noen av disse behandlingene av lege?

	Ja	Nei	Vet ikke
Lokalbehandling (f.eks. kremer eller oppløsninger)			
Antibiotika tabletter (f.eks. Tetracyclin)			
Roaccutan tabletter			

92) Har du eller har du noen gang hatt psoriasis?

Ja Nei Vet ikke

Hvor mye plaget er du av psoriasis i dag?
Svar på en skala fra 0-10, der 0 tilsvarer ingen plager og 10 tilsvarer verst tenkelige plager.

0 1 2 3 4 5 6 7 8 9 10

Verkebyller er svært store kviser som er ømme/smertefulle og som ofte gir arr.

94) Har du noen gang hatt verkebyller under armene/armhulene?

Ja
Nei
Vet ikke

95) Har du noen gang oppsøkt lege pga verkebyllene?

Ja Nei

96) Har du noen gang hatt verkebyller i lyskene/nært skrittet?

Ja
Nei
Vet ikke

97) Har du noen gang oppsøkt lege på grunn av verkebyllene?

- Ja
- Nei

98) Har en lege noen gang sagt at du har...

	Ja	Nei	Vet ikke
høysnue eller neseallergi?			
astma?			
barneeksem eller atopisk eksem?			

SMERTER

99) Har du langvarige eller stadig tilbakevendende smerter som har vart i 3 måneder eller mer?

- Ja
- Nei

100) Hvor lenge har du hatt disse smertene? (Dersom du har flere typer smerte, svar for den som har vart lengst)

- 3 - 6 måneder
- 6 - 12 måneder
- 1-2 år
- 3-6 år
- Mer enn 6 år

101) Hvor ofte har du vanligvis disse smertene?

- Hele tiden, uten opphør
- Hver dag, men ikke hele tiden
- Hver uke, men ikke hver dag
- Sjeldnere enn hver uke

Hvor er det vondt?
(kryss av på alle aktuelle steder)

	Venstre side	Høyre side
Skulder	☐	☐
Arm/albue	☐	☐
Hånd	☐	☐
Hofte	☐	☐
Lår/kne/legg	☐	☐
Ankel/fot	☐	☐

	Midten
Hode/ansikt	☐
Kjeve/kjeveledd	☐
Nakke	☐
Øvre del av ryggen	☐
Korsryggen	☐
Bryst	☐
Mage	☐
Underliv/kjønnsorganer	☐

104) Hva mener du er årsaken til smertene? (flere svar mulig)

- [ ] PC-bruk, dataspill og lignende
- [ ] Idrettsskade
- [ ] Ulykke/skade
- [ ] Kirurgisk inngrep/operasjon
- [ ] Migrene/hodepine
- [ ] Medfødt sykdom
- [ ] Tannproblemer
- [ ] Whiplash
- [ ] Prolaps (skiveutglidning i ryggen)
- [ ] Annet ryggproblem
- [ ] Nerveskade
- [ ] Mage- eller tarmsykdom
- [ ] Annet, spesifiser her
- [ ] Vet ikke

Hvis du har langvarige smerter flere steder i kroppen, gjelder de 4 neste spørsmålene smerten som plager deg mest.

Hvor sterke vil du si at smertene vanligvis er?
Svar på en skala fra 0-10, der 0 tilsvarer ingen smerte og 10 tilsvarer verst tenkelig smerte.

Dersom du har flere typer smerte, svar den som plager deg mest.

○ 0 ○ 1 ○ 2 ○ 3 ○ 4 ○ 5 ○ 6 ○ 7 ○ 8 ○ 9 ○ 10

Hvor sterke er smertene når de er på sitt sterkeste?
Svar på en skala fra 0-10, der 0 tilsvarer ingen smerte og 10 tilsvarer verst tenkelig smerte.

Dersom du har flere typer smerte, svar den som plager deg mest.

0 1 2 3 4 5 6 7 8 9 10

I hvor stor grad påvirker smertene søvnen din?
Svar på en skala fra 0-10, der 0 tilsvarer ingen smerte og 10 tilsvarer verst tenkelig smerte.

Dersom du har flere typer smerte, svar den som plager deg mest.

0 1 2 3 4 5 6 7 8 9 10

I hvor stor grad hindrer smertene deg i å utføre vanlige aktiviteter hjemme og på skolen?
Svar på en skala fra 0-10, der 0 tilsvarer ingen smerte og 10 tilsvarer verst tenkelig smerte.

Dersom du har flere typer smerte, svar den som plager deg mest.

0 1 2 3 4 5 6 7 8 9 10

MAGE- OG TARMPROBLEMER

109) I løpet av de siste 2 månedene: Hvor ofte har du hatt smerte eller ubehag i magen?

- Aldri
- 1-3 ganger i måneden
- En gang i uka
- Flere ganger i uka
- Hver dag

110) Hvor lenge har du vært plaget av smerte eller ubehag i magen?

- Mindre enn 1 måned
- 2 måneder
- 3 måneder
- 4-11 måneder
- Ett år eller mer

111) I hvilken del av magen er det du har hatt smerte eller ubehag? (kryss av for alt som passer)

- [ ] Over navlen
- [ ] Rundt navlen
- [ ] Nedenfor navlen

112) Når du har smerter eller ubehag i magen, hvor lenge varer det vanligvis?

- ○ Mindre enn 1 time
- ○ 1-2 timer
- ○ 3-4 timer
- ○ Mesteparten av dagen
- ○ Hele døgnet

Når du har smerte eller ubehag i magen, hvor sterke smerter har du vanligvis?
Svar på en skala fra 0-10, der 0 tilsvarer ingen smerte og 10 tilsvarer verst tenkelig smerte.

Dersom du har flere typer smerte, svar den som plager deg mest.

○ 0 ○ 1 ○ 2 ○ 3 ○ 4 ○ 5 ○ 6 ○ 7 ○ 8 ○ 9 ○ 10

114) Når du har smerter eller ubehag i magen, hvor ofte blir det bedre etter at du har hatt avføring?

- ○ Sjelden eller aldri
- ○ En del ganger
- ○ For det meste/hver gang

115) Når du har smerter eller ubehag i magen, hvor ofte skjer det i forbindelse med at du..

	Sjelden eller aldri	En del ganger	For det meste
har fastere eller mer klumpete avføring enn vanlig?	○	○	○
har løsere eller mer vannaktig avføring enn vanlig?	○	○	○
hadde avføring oftere enn vanlig?	○	○	○
hadde avføring sjeldnere enn vanlig?	○	○	○

HODEPINE

116) Har du vært plaget av hodepine det siste året?

○ Ja ○ Nei

117) Hva slags hodepine er du plaget av? (Du kan sette flere kryss)

☐ Migrene ☐ Annen hodepine ☐ Vet ikke

118) Omtrent hvor mange dager per måned har du hodepine?

○ Mindre enn 1 dag
○ 1-6 dager
○ 7-14 dager
○ Mer enn 14 dager

119) Er hodepinen vanligvis:

	Ja	Nei
Bankende/dunkende smerte	○	○
Pressende smerte	○	○
Ensidig smerte (høyre eller venstre)	○	○

120) Hvor lenge varer hodepinen vanligvis?

- Mindre enn 4 timer
- 4 timer - 1 døgn
- 1-3 døgn
- Mer enn 3 døgn

121) Før eller under hodepinen, kan du da ha forbigående:

	Ja	Nei
Synsforstyrrelse? (takkede linjer, flimring, tåkesyn, lysglimt)		
Nummenhet i halve ansiktet eller i hånden?		
Forverring ved moderat fysisk aktivitet?		
Kvalme og/eller oppkast?		

122) Hvor ofte pusser du vanligvis tennene dine? (sett ett kryss)

- Sjeldnere enn 1 gang per uke
- 1 gang per uke
- 2-3 ganger per uke
- 4-6 ganger per uke
- 1 gang daglig
- 2 eller flere ganger daglig

Hvor smertefullt, jevnt over, synes du det er å gå til tannlegen?
Svar på en skala fra 0-10, der 0 tilsvarer ingen smerte og 10 tilsvarer verst tenkelig smerte.

0 1 2 3 4 5 6 7 8 9 10

Nedenfor er det fire spørsmål om hvordan du opplever det er å gå til tannlege. Les hvert spørsmål og velg det svaralternativet som du synes passer best for deg.

124) Dersom du skulle gå til tannlegen i morgen, hva ville du føle?

- Jeg ville se frem til det som en ganske hyggelig opplevelse
- Det ville være det samme for meg, ikke bety noe
- Det ville gjøre meg litt urolig
- Jeg ville bli redd for at det skulle bli ubehagelig og vondt
- Jeg ville bli svært redd med tanke på hva tannlegen kanskje skulle gjøre

125) Når du venter på tannlegens venteværelse, hvordan føler du deg da?

- Avslappet
- Litt urolig
- Anspent, nervøs
- Redd, engstelig
- Så redd at jeg av og til begynner å svette eller nesten føler meg syk

126) Når du sitter i tannlegestolen og venter på at tannlegen skal begynne behandlingen, hvordan føler du deg da?

- Avslappet
- Litt urolig
- Anspent, nervøs
- Redd, engstelig
- Så redd at jeg av og til begynner å svette eller nesten føler meg syk

Tenk at du sitter i tannlegestolen og skal få tennene renset og pusset. Mens du sitter og venter på at tannlege skal finne frem instrumentene som brukes til å skrape og pusse med,

127) hvordan føler du deg da?

- Avslappet
- Litt urolig
- Anspent, nervøs
- Redd, engstelig
- Så redd at jeg av og til begynner å svette eller nesten føler meg syk

128) Har du øresus?

- Aldri
- Sjelden
- Ofte

129) Hvor ofte har du øresus?

- Hele tiden, uten opphør
- Hver dag, men ikke hele tiden
- Hver uke, men ikke hver dag
- Sjeldnere enn hver uke

130) Hvor lenge varer vanligvis periodene med øresus?

- Mindre enn 10 minutter
- 10 minutter - 1 time
- Mer enn 1 time

131) Når får du vanligvis øresus?

- Etter sterke lyder
- Når det er stille
- Vet aldri når

Noen bryr seg ikke om lyden, for andre oppleves det svært plagsomt å ha øresus. Angi hvor plaget du er av øresusen.
Svar på en skala fra 0 til 10, der 0 tilsvarer ingen plager og 10 tilsvarer verst tenkelige plager.

- 0
- 1
- 2
- 3
- 4
- 5
- 6
- 7
- 8
- 9
- 10

133) På hvilket øre har du vanligvis øresus?

- Bare høyre
- Bare venstre
- Begge, men mest høyre
- Begge, men mest venstre
- Like mye på begge

134) Omtrent hvor gammel var du når du begynte å ha øresus ofte?

Velg...

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FORHÅNDSVISNING

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PUBERTET

28) Når man er tenåring, er det perioder da man vokser raskt. Har du merket at kroppen din har vokst fort (blitt høyere)?

- Nei, den har ikke begynt å vokse
- Ja, den har såvidt begynt å vokse
- Ja, den har helt tydelig begynt å vokse
- Ja, det virker som om jeg er ferdig med å vokse raskt

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FORHÅNDSVISNING

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29) Og hva med hår på kroppen (under armene og i skrittet)? Vil du si at håret på kroppen din har:

- Ikke begynt å vokse enda
- Såvidt begynt å vokse
- Helt tydelig begynt å vokse
- Det virker som om håret på kroppen er utvokst

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FORHÅNDSVISNING

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30) Hvor gammel var du da du begynte å få hår i skrittet (kjønnshår)?

Velg ... Velg ...

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FORHÅNDSVISNING

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31) Har du begynt å komme i stemmeskifte?

- Nei, har ikke begynt ennå
- Ja, har såvidt begynt
- Ja, har helt tydelig begynt
- Det virker som om stemmeskifte er ferdig

32) Har du begynt å få bart eller skjegg?

- Nei, har ikke begynt ennå
- Ja, har såvidt begynt
- Ja, har helt tydelig begynt
- ja, har fått en god del skjeggvekst

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