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Adolescents´ lifestyle and bone health

The Tromsø Study, *Fit Futures*

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Anne Winther

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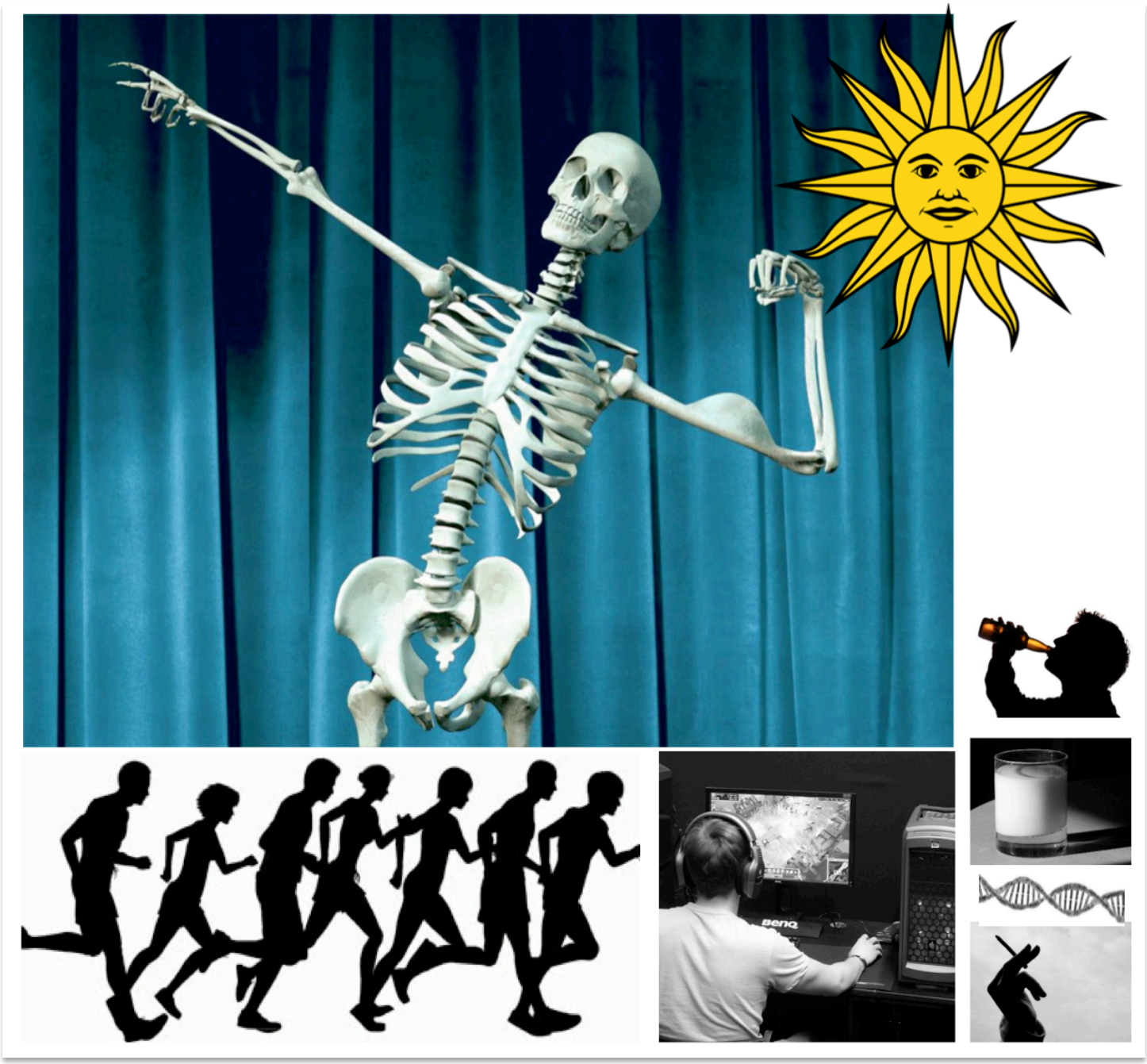

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Table of Contents

Summary

Background: Osteoporotic fractures constitute a major economic burden for communities and health care sectors in western societies. For most individuals, following osteoporotic fractures is reduced quality of life, increased morbidity and mortality, in addition to the monetary costs. The incidence of osteoporotic fractures in Norway is among the highest in the world. Bone strength is an important predictor of fracture risk throughout life, and in the elderly, both peak bone mass and subsequent bone loss are important determinants for the risk of osteoporotic fractures. The foundation of bone strength is laid during growth in childhood and adolescence, before the bone mass peaks. Adolescence is a time of great vulnerability and we know little about how current lifestyle and behavioural pattern influences bone health and bone mass distribution in adolescents.

Objectives: The main objective of the present PhD-project was to describe Norwegian adolescents´ bone mass levels, to compare these with international reference values, and to explore relationships between potential modifiable lifestyle factors and bone mass.

Methods: The cross-sectional study, *Fit Futures* is an extension of the population-based Tromsø Study. In 2010/2011, all first year upper-secondary school students in the municipalities of Tromsø and Balsfjord were invited to this extensive multipurpose health survey. A total of 1,038 adolescents aged 15-18; 508 girls and 530 boys, attended the survey, providing an attendance rate close to 93%. We measured hip and total body bone mineral density (BMD), as well as body composition, i.e. distribution of fat (FM) and muscle mass (LM) by DXA (Lunar prodigy). Weight and height were measured and body mass index (BMI) calculated. Information on lifestyle variables was collected through interviews and questionnaires, and blood samples were drawn.

Results: For the 16-years old, BMD values were significantly higher than the reference values provided by Lunar. Physical activity and BMI was highly associated with BMD levels, in addition smoking was negatively associated with BMD in boys. Boys reported more hours spent on TV/computer use, and we observed persisting inverse relationships between this sedentary pastime activity and BMD levels among boys. Body composition was strongly related to BMD; the importance of LM was higher than FM. However, the

associations of high LM levels with BMD were observed more prominently in boys. In adolescents with lower LM, high FM seemed to ameliorate the effect of deficient LM. **Conclusion:** Norwegian adolescents have similar or probably higher BMD levels compared to age-matched European peers. Modifiable life style factors like physical activity and BMI seems to be the most prominent predictors for BMD. We observed a gender specific difference, as lifestyle factors explained more of the variance in BMD-levels in boys. Low lean mass and lower physical activity levels attribute an adverse influence on bone health, especially among boys, whereas BMD levels in sedentary girls seem to be protected by their habitual fat mass.

List of papers

The following papers are part of the thesis:

Paper I.
Winther A, Dennison E, Ahmed LA, Furberg A-S, Grimnes G, Jorde R, Gjesdal CG, Emaus N. The Tromso Study: *Fit Futures*: a study of Norwegian adolescents' lifestyle and bone health. *Arc Osteoporos* 2014;**9**(1):185

Paper II.
Winther A, Ahmed LA, Furberg A-S, Grimnes G, Jorde R, Nilsen OA, Dennison E, Emaus N. Leisure time computer use and adolescent bone health - findings from the Tromsø Study, *Fit Futures*: a cross-sectional study. *BMJ Open* 2015; **5**; (6)

Paper III.
Winther A, Jørgensen L, Ahmed LA, Christoffersen T, Furberg A-S, Grimnes G, Jorde R, Nilsen OA, Dennison E, Emaus N. Bone mineral density at the hip and its relation to fat mass and lean mass in adolescents. The Tromsø Study, *Fit Futures*. (Submitted, 2015).

Abbreviations

ANOVA: analysis of variance

ANCOVA: analysis of covariance

BMC: bone mineral content

BMD: bone mineral density

BMD_{FN}: BMD femoral neck

BMD_{TB}: BMD total body

BMD_{TH}: BMD total hip

BMI: body mass index

CHC: combined hormonal contraceptives

CI: confidence interval

CV: coefficient of variation

DXA: dual-energy x-ray absorptiometry

FF1: *Fit Futures 1,* 2010/2011

FF2: *Fit Futures 2,* 2012/2013

FM: fat mass

FN: femoral neck

LM: lean mass

NIPH: Norwegian Institute of Public Health

OR: odds ratio

PBM: peak bone mass

PDS: Pubertal Development Scale

ScTWdays: screen time spent during weekdays

ScTWends: screen time spent during weekends

SD: standard deviation

SPSS: Statistical Package for the Social Sciences

TB: total body

TH: total hip

UiT: UiT The Arctic University of Norway

UNN HF: University Hospital of North Norway

WHO: World Health Organisation

1. Introduction

The annually 9,000 hip fractures and 15,000 wrist fractures recorded in Norway [1, 2] is a case for major health concern. Is there a possible way to prevent some of these?

1.1 Epidemiology of osteoporosis and fractures

1.1.1 Definition of osteoporosis

Back in 1993 the Consensus Development Conference gathered an international expert panel, to discuss the nature of osteoporosis. The group stated that: *"Osteoporosis is a systemic skeletal disease characterized by low bone mass and micro-architectural deterioration of bone tissue, with a consequent increase in bone fragility and susceptibility to fracture"* [3]. The condition of osteoporosis, clinically manifested by fragility fractures, most common at the distal forearm, spine and hip, constitute a major public health challenge worldwide.

1.1.2 Burden of osteoporosis

Osteoporotic fractures in the elderly imply a huge economic impact; in EU the total costs in 2010 were stipulated to 37 billion Euros [4]. Also Sweden and Denmark report substantial societal expenditures, with direct costs to medical care and community care, as well as indirect costs and loss of quality-adjusted life-years (QUALY) [5, 6]. In Norway we have no updated estimates of the annual expenses of osteoporotic fractures. Based on Danish figures Dahl in a recent PhD-thesis [7], calculated the Norwegian expenditures to 10 billion NOK. In addition to monetary costs, adverse outcomes of osteoporotic fractures are related to increased mortality and morbidity [8], with the hip fractures as the most serious. Former calculation suggested that about 10-20% more women than expected for their age die after suffering a hip fracture [9]. Recent data from Norway concluded that 21% of women and 33% of men died within the first year after hip fracture [10]. The risk of institutionalisation increases as one third of individuals who have sustained a hip fracture become totally dependent afterwards [9, 11].

1.1.3 Fracture rates

The incidence of osteoporosis differs among populations and ethnic groups, and the

incidence of fractures differs between countries [8, 12-14]. Norway has one of the highest hip fracture incidences in the world ever reported; age-adjusted fracture rates per 10.000 for the age group ≥ 50 in 1978/79 were calculated to 104.5 and 35.8 for women and men, respectively. The incidence rose to 124.3 and 44.9 in 1988/89, and then declined to 118.0 and 44.0 in 1996/97 [15]. A considerable increase in incidence with age was observed in both genders [15]. A recent review addressing secular trends states that hip fracture rates increased through the second half of the last century in Western populations (America, Europe and Oceania), although rates appear to have stabilized and age-adjusted decreases are reported during the last two decades [13]. Moreover, the trends seem to be similar for forearm fractures [13]. Also in Norway such trends are observed; there were no increase in hip fracture rates between 1994-96 and 2006-08 in the Harstad Injury Prevention Study [16]. Furthermore, the age-standardized total incidence of hip fracture decreased by 13.4% in women and 4.8% in men between 1999 and 2008 [2]. However, as the Norwegian population and the life expectancy are increasing [17] it is likely that the population at risk and the absolute numbers of fractures will still rise [18].

The explanation of the decreasing fracture incidence is unknown, but is likely to be multi factorial as several determinants have been linked to BMD-levels, osteoporosis and fracture risk. This may also reflect cohort effects driven by improved nutritional status during childhood and growth in the present elderly. As this is merely speculation from ecological studies, we need more knowledge on how lifestyle affects bone health in younger age groups.

1.2 Bone biology and physiology

1.2.1 Bone as a tissue

The skeletal system is a complex and dynamic body, composed of different tissues working together. The skeleton includes 208 bones; the skull with its tiny knuckles, the vertebral column and the thorax are denoted the axial skeleton, while upper and lower limbs together with the shoulder and pelvic girdle constitute the appendicular skeleton. The task of this structural framework is to support soft tissues, protect internal organs, and together with the muscles perform movements. Inside the knuckles the red bone

marrow produces blood cells, the yellow bone marrow is storage of triglycerides and the bony tissue serves as a reservoir for several minerals, especially for calcium and phosphorus in maintaining the mineral homeostasis [19].

1.2.2 Components of bone

The adult skeleton consists mainly of compact bone tissue, the cortical bone (80%). The osteon is the structural unit composed of concentric layers around the central Haversian canal, with its blood vessels, nerves and lymphatic vessels [19]. An envelope of cortical bone, comprising series of cylindrical units denoted the Haversian systems, surrounds all bones of the skeleton. A membrane, the periosteum covers the outer surface of the cortical bone [20]. Close to the marrow cave a corresponding membranous structure, the endosteum, covers the inner surface of the cortical bone [19, 20].
In the spongy or trabecular bone (20%), the osteons are called packets. These are semi lunar in shape and composed of concentric lamellae. This bone tissue is cancellous and characterized by bony trabecular plates interspersed spaces filled with bone marrow. This type of bone is light and can withstand compressive loads better than cortical bone, because it can absorb energy more efficiently [21]. The bone tissue contains of a composite of a cellular network and extracellular matrix. The bone matrix consists of 85% collagen, and minerals such as calcium and phosphate are deposited in the bone matrix as hydroxyapatite crystals [20].

In bone there are four main types of cells. The **Osteogenic** cells are bone marrow stem cells. By cell division the resulting cells develop into osteoblasts [19]. **Osteoblasts** synthesize and secrete collagen fibres to build extracellular matrix of bone fibres. Buried in this extracellular matrix they differentiate into osteocytes. Another key role is to promote osteoclast differentiation by secreting cytokines [21]. **Osteocytes** are cells of communication and are the main cells in bone tissue, covering the bone surface. They detect and transduce the effects of mechanical loading. By detection of micro damage they co-ordinate the subsequent bone remodelling and maintain the daily metabolism within bone [22]. The **osteoclasts** concentrated in the endosteum derive from monocytes. The formation and activation of these cells are dependent of local cytokines from the

osteoblasts and other cells. Their main task is resorption; to break down and remove the extracellular matrix [23].

1.2.3 Bone growth and development

About 8 weeks of embryotic development, the pattern of the skeleton has been largely determined. This early "skeleton" is the site for ossification, the initial bone formation that starts 6 weeks post fertilization [24]. The majority of bones commence ossification during the first weeks of fetal stage, while others several weeks after birth. The fetal stage is characterized by rapid growth in bone size and maintenance of the bone´s shape, which in turn prerequisite adequate maternal nutrition and fetal movements [24]. After birth, the skeleton maintains a fast rate of growth in length and thickness. The long bones continue their growth during infancy, childhood and adolescence until they reach their adult size. Growth in length is driven by bone formation on the diaphyseal side of the epiphyseal plate, while increased thickness is related to net bone formation on the periosteal surface. Combined with resorption on the endosteal surface, the medullar cavity enlarges [19]. Throughout life, each bone undergoes continuous modelling and remodelling to adapt to changing biomechanical forces, and to remove and replace old damaged bone in order to maintain bone strength [20].

1.2.4 Development of skeletal sex differences

Through childhood, growth in stature is determined by an appendicular growth rate approximately twice the rate of the axial growth. At puberty the appendicular growth rate slows down as the axial growth rate accelerate [25]. During the first two years of puberty the rates of these processes are the same. Onset of puberty is approximately 2 years earlier in girls; this gives the boys two additional years of appendicular growth resulting in different morphology [25].

During puberty in boys, the periosteal apposition increases the bone width and the endosteal resorption expands the inner cavity. As the rate of the external bone formation is higher than the endosteal bone resorption, the cortical thickness increases. In girls the rate of periosteal apposition decreases, whereas there are no changes, at some sites even a

narrowing, of the medullar cavity. This leads to smaller total and medullar size, with a similar cortical thickness compared to boys [25].The greater periosteal expansion in boys compared to girls has been attributed to higher pubertal levels of androgens in male [26].

Low levels of oestrogens and perhaps androgens are responsible for the linear growth spurt in both sexes at the beginning of puberty [26]. As the rate of linear growth peaks earlier than the bone mass acquisition, there will be a "transitory phase of porosity" [25] which is linked to increased fracture rates in girls 10-12 and boys 12-14 years of age [27]. At the end of puberty high levels of oestrogens promotes closure of the epiphysis and the longitudinal growth slows down [26]; cortical porosity decreases as bone formation increases at the trabecular surface [25]. Young males seem to develop thicker but similar numbers of trabeculae compared to girls, and this sex difference may be of importance for fracture risk in later life, as thin trabeculae are more easily perforated [25].

1.2.5 Bone strength

The strength of the bone is defined by the amount of bone mass, by external size and shape (elliptical, external diameters), as well as the internal structure of the cortical and trabecular bone [25, 28]. To maintain a light but strong bone the cortical thickness and porosity, the numbers of trabeculae, their thickness, orientation and connectivity is organized to minimize mass and optimize strength [29, 30]. The quality of the bone matrix is also of importance as it regulates the bone mineralization [20, 31].

1.2.6 Measuring bone strength

Several non-invasive techniques, for research as well as for clinical purposes, have been developed for *in vivo* prediction of bone strength. Quantitative computed tomography (QCT) assesses bone density by 3 dimensions (3D) images. The QCT technique provides robust measurements of geometry and volumetric bone density in trabecular and cortical compartments at sites that are most prone to fractures and allows adaptions in density and geometry to be distinguished [32, 33]. The more resent high resolution peripheral QCT (HR-pQCT), is a technique able to capture both density and structure measurements at an excellent precision (2-4%) [33]. Opposed to the CT imaging, the magnetic resonance

imaging (MRI) technique allows 3D measurement of bone geometry and microarchitecture without ionizing radiation [33]. Despite these approach´s ability to perform structural images of high quality, the techniques are not widely used. They require specialised scanners included extensive resources and soft ware, and the measurements are limited to the appendicular skeleton.

However, for almost 20 years a low ionizing radiation examination of bone mineral content has been regarded as a good and applicable surrogate measure of bone strength. The bone mineral content (BMC) divided by its scanned area; the areal bone mineral density (BMD), measured by dual energy x-ray absorptiometry (DXA) (Figure 1) predicts 60-70 percentages of the variance in bone strength [31]. A strong relationship between BMD levels and the probability of fracture has been documented [34, 35]. The DXA approach is considered as the gold standard for diagnosis of osteoporosis, as reference values based on DXA measurements define the diagnostic criterion [36]. However, the World Health Organisation (WHO) diagnostic categories for normal BMD-values, osteopenia, and osteoporosis, based on BMD T-scores are not recommended used before the age of 20, as they are not applicable to children and adolescents who have not reached peak bone mass [37, 38].

Figure 1. Measurement of total body bone mineral density using dual energy x-ray absorptiometry (DXA)

1.3 *Peak bone mass and its determinants*

1.3.1 Definition of peak bone mass

Bone mass increases as the skeleton grows, reaching a plateau, the peak bone mass (PBM) at the end of the second or early in the third decade of life [39]. PBM are defined as the amount of bone tissue present at the end of skeletal maturation [40]. Approximately 70-80% of the variance in PBM in a population is determined by genetic factors [41, 42]. To achieve the full genetic potential for bone strength, sufficient nutrients and optimal skeletal loading are required [42]. Interruption of normal physiological processes by illness or lifestyle may lead to a degree of bone deposition, which is below expected level for an individual, given its genetic constitution. This thesis focuses on the modifiable determinants of PBM.

1.3.2 Weight and nutrition

Body weight that is a compound of hereditary and environmental factors, is the largest single determinant of the variability in adult bone mass. Body weight explains roughly half of the bone mass´ variance at a population level [41]. The positive influence of body mass index (BMI) on bone mass is well established in adults as well as in youths [43-46], and weight maintenance has been regarded as protective of future fracture risk [47]. However, some studies suggest that the positive effect of BMI is limited to a certain threshold [48, 49]. In addition to the loading effect of body weight, non-mechanical factors such as bone active hormones from the adipocyte [50], the muscle [51] and the gut [52] may have an anabolic impact on bone. As the adverse effects of obesity in children and adolescents´ general health are of major concern [53, 54], study of weight and body composition´s influence on bone need further elaboration.

Beyond sufficient energy intake, a broad array of macro- and micronutrients are important for bone health. Some key nutrients such as calcium, vitamin D and proteins, must be present in adequate levels. For the collagen synthesis, enough proteins are of importance [55]. Low calcium levels seem to be a limiting factor for bone accumulation,

however above a certain threshold the bone acquisition remains constant [56]. Active calcium absorption through the gut depends on sufficient vitamin D levels. Vitamin D is generated when the skin is exposed to sunlight. At higher latitude as Tromsø, at approximately 70 degrees northern latitude, the sun is too low for cutaneous vitamin D synthesis between October and March [57], and sufficient vitamin D levels are in general based on diet. However, vitamin D deficiency is common among children and adolescents [58] and also described in our cohort [59]. An optimal development of bone requires a diet rich in all essential nutrients. Diary products are preferable, as this food group provides 20-75% of the recommended calcium, protein, phosphorus, magnesium and potassium intakes [55]. Milk avoidance appears to have deleterious effects on children´s bone, and the effects of low milk consumption in childhood may extend into adult life. Also carbonated soft drinks have been related to decreased BMD in girls, and increased fracture risk in both sexes. Whether this effect is due to milk replacement are unclear, as some studies suggest independent effects of these beverages [58]. How these key nutrients influence bone acquisition in a high-income Western adolescent population would be important to clarify.

1.3.3 Physical activity

In addition to the genetic constitution and nutritional status, mechanical loading modifies the skeleton. High impact sports, participation in recreational play, as well as higher levels of normal physical activity have a positive effect on bone strength [32]. Regular weight bearing exercises with dynamic activities including high strain rates, such as jumping and running are widely reported to be “osteogenic” [60, 61]. Physical activity divided into short bouts with rest periods in between, is regarded to be the most beneficial as the sensitivity of the bone cell to loading stimulus returns after a period of rest [39]. The peak momentary muscle forces as described by Frost´s “Mechanostat theory” have a strong influence on bone acquisition in youth [62, 63]. During growth when the periosteal surfaces have a greater proportion of active bone cells, the mechanical loading increases the already active modelling process [32]. Skeletal adaptation to loading implies changes

in bone mass, geometry and structural properties, as well as material properties. The trade-off between strength and mass implies that a small amount of bone is strategically placed away from the axis of bending (Figure 2), where it has an exponential effect to resist bending loads [28, 30, 32].

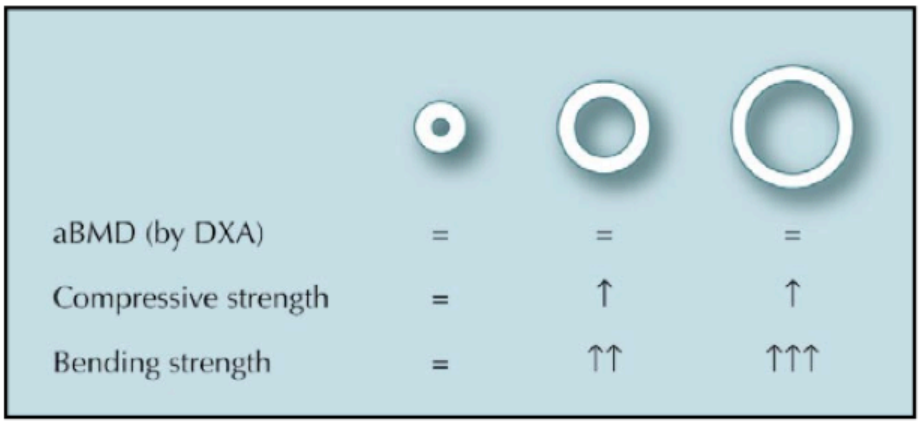

Figure 2. Effect of cross-sectional geometry on long-bone compressive and bending strength. Arrows indicate increased strength compared with the left-hand structure. Reprinted with permission from Mary L. Bouxsein

The longitudinal effects of physical activity during childhood and adolescence on bone health and future fracture risk are not fully explained. There are indications of the pre-pubertal and early puberty as the most beneficial periods for bone acquisition and that the acquired bone mass is maintained into adulthood [39, 42, 64]. A recent study concludes that physical activity during youth should be encouraged for lifelong bone health, due to its effects on bone size and strength rather than its effect on bone mass [65]. Mapping out the physical activity levels and the influence on bone in this youth cohort, provide data for further investigations on long-term effects of physical activity in a public health perspective.

1.3.4 Tobacco and alcohol

In a preventive perspective, adverse effects on PBM have also been examined. Adolescent tobacco use is associated with lower BMD-levels in some, but not all studies [38, 41, 66]. The contradictory findings may be attributed to differences in smoking habits and the ability to control for confounding factors. In the adult population, several underlying pathophysiologic mechanisms that predispose smokers to bone loss have been defined. The deleterious effects on fracture risk imply all skeletal sites through BMD-dependent as well as BMD-independent factors. The adverse effects are influenced of dose and duration, and some studies have observed a reversible effect of cessation [67]. Figures from Norhealth [68] indicate increased snuffing habits between 2005 and 2009 among Norwegians aged 15, while traditional smoking decreases. The effect of snuffing on bone is hardly described in the literature. A small study of elderly women, for whom smokeless tobacco use from childhood was common, reported that such tobacco use had negative impact by increasing BMD loss with age [69]. To my knowledge, potential long-term effects on bone of such a notable change in tobacco use are not described at a population level.

The effects of alcohol have been extensively studied in adults. The results indicate that excess alcohol consumption is associated with low bone density, mainly by suppression on bone formation [41]. Another systematic review and meta-analysis concluded that most evidence support a beneficial effect of moderate alcohol consumption on bone density [70]. However, the effect of alcohol intake on peak bone mass is unclear [38, 41], and results from this study population may be of value.

1.3.5 Hormonal contraceptives

Oestrogens are essential for the pubertal bone changes in boys and girls. The positive effects on PBM and subsequently on bone loss have been demonstrated both in men and women [71]. Hormonal contraceptives are commonly used in early life, within the first reproductive years, for contraceptive as well as non-contraceptive purposes [72, 73]. The

most frequently prescribed, combined hormonal contraceptives (CHC), promote a reduction of oestrogen and a suppression of endogenic progesterone production by the ovaries, so that the circulating levels of sex hormones are dose dependent of the CHC-therapy [72]. Several studies have investigated the effect of CHC, and evidence suggests that the effect is different in young women who have not yet achieved PBM, compared to skeletally mature women. Depending on oestrogen dose and duration of exposure, CHC may interfere with the normal acquisition of peak bone mass in adolescents [72-74]. Progestin-only methods are available as pills, injections and implants. While pills contain a small dosage of contraceptive agents, an intramuscular or subcutaneous depot injection provides a higher dose of progestin, which has been linked to BMD loss in adolescents [75]. Thus, the possible influence of these contraceptives is important to follow when bone health is studied.

2. Rationale and aims

BMD in the elderly is a function of the amount of bone gained during growth and the rate of subsequent bone loss during aging [76]. In adolescence bone mass and size varies to a great extend around the age-specific means, estimated to 10-15% (1SD), while 1 SD variance in the rate of bone loss in the elderly is estimated to approximately 1% [25]. Preventive strategies have focused on the age-related bone loss, and the frequency and severity of fractures among the elderly. There is however, a growing understanding for the importance of bone mass during growth as a compensation for the later inevitable bone loss [30, 77]. As the differences in achievement of bone mass during growth is greater than the rates of loss during ageing, it is likely that trait variances established at the completion of growth are more important for future fracture risk. Moreover, factors that modify skeletal morphology, like exercise and nutrition are probably best instituted during growth [25, 65].

For the first time in Norway, the *Fit Futures* study provided a unique opportunity to explore the association between lifestyle and bone health at the entrance of adult life. The main objective of the present PhD-project was to identify young people´s health behaviour, which may influence the acquisition of PBM and by that: bone strength at the entrance of adult life. The differences between the genders were explored and the following three main issues have been addressed and represented as one paper each.

1. Description of bone mineral density levels in Norwegian adolescents compared with international reference values, and identification of modifiable predictors, which may influence the acquisition of peak bone mass at the femoral sites.
2. Elaboration of a possible adverse effect of sedentary lifestyle on bone health, focused on increasing time spent in front of television and computers in leisure time.
3. Exploring the influence of BMI and body composition on adolescents´ bone mass density at the hip.

3. Materials and methods

3.1 Study population: Fit Futures

The Tromsø Study [78] is a population-based study with repeated health surveys in the municipality of Tromsø inviting all residents in specific age groups. *Fit Futures* is an expansion of the Tromsø Study, in collaboration with the University Hospital of North Norway (UNN HF), UiT The Arctic University of Norway and the Norwegian Institute of Public Health (NIPH), with the intension to collect data from age groups sparsely represented in population-based studies. The survey is the start of an extensive, multipurpose longitudinal health survey. The overall aim of *Fit Futures* is to study adolescents´ health and health behavior in a broad perspective. In total, 13 research groups are represented with projects in the first wave of *Fit Futures*. The research groups are responsible for their respective data collection, and information from the general clinical examinations, interviews and questionnaires are shared among the groups. For this thesis we had access to bone measurements in addition to body composition, height and weight. We had information from questionnaires about puberty, about lifestyle variables such as physical activity, sedentary behaviour, smoking and snuffing habits, alcohol consumption, and nutritional information on daily calcium and soft drinks consumption. Finally, analyses from collected blood samples provided serum vitamin D-levels, while information on past medical history, use of medication and contraceptives was obtained by interviews.

In 2010/2011 all first year upper-secondary school students in the two neighbouring municipalities, Tromsø and Balsfjord were invited to the cross-sectional *Fit Futures 1* (FF1). In this geographical area (Figure 3) there were eight schools representing an urban as well as a rural population.

Figure 3 Geographical areas for the Tromsø Study, *Fit Futures*

The invited cohort included 1,117 participants mainly aged 15 – 19 years old, of which 1,038 adolescents attended the survey, providing an attendance rate close to 93 %. The main analyses are based on data from adolescents younger than 18 years of age at FF1 (n=961). However, numbers of participants in each paper varies according to variables of interest and complete datasets. A second wave of the survey *Fit Futures 2* (FF2), carried out in 2012/2013 provided repeated BMD-measurements of 66% of the original cohort, which are included in paper II.

3.2 Ethics

The Norwegian Data Protection Authority (reference number 2009/1282) and The Regional Committee of Medical and Health Research Ethics (2011/1702/REK nord) approved the study in July 2010 and October 2011, respectively. According to the

Declaration of Helsinki [79] and the Health Research Act [80] informed consents were provided. All participants signed a declaration when arriving at the study site. Juvenile participants aged 16 and older, in line with the Health Research Act (§17). Younger participants regarded incompetent to consent, had to bring written permission from their guardians, as described by guidelines in the Patients´ Rights Act [81].

3.3. Measurements

3.3.1 BMD measurements

By DXA (GE Lunar Prodigy, Lunar Corporation, Madison, Wisconsin, USA) with enCORE paediatric software version 13.4 [82], we measured BMD (g/cm^2) at total hip, femoral neck and total body sites, as well as total body lean mass (LM) (g) and total body fat mass (FM) (g). Specially trained research technicians at UNN performed all scans at the same device according to the protocol provided by the manufacturer. They reviewed and reanalysed the scans if necessary, and a final quality control excluded ten scans, due to artefacts (metal in pockets etc.). We included BMD-measurements from the left hip by default, or from the right hip in cases of poor quality scans.

3.3.2 Anthropometric measurements

We measured height to the nearest 0.1 cm and weight with a precision of 0.1kg on an automatic electronic scale, the Jenix DS-102 stadiometer (Dong Sahn Jenix co., Ltd., Seoul, Korea), according to standardised procedures in The Tromsø Study. BMI was calculated as weight (kg) and divided by squared height (m^2), and categorised according to Cole et al.´s BMI cut-off points for children and adolescents [83, 84] and the WHO index for those older than 18 [85]. The categories were used to describe the study population in paper I, whereas further analyses were made of the continuous BMI-variable.

3.3.3 Vitamin D-levels

Non-fasting blood was obtained, and serum 25(OH)D was analysed in stored sera, using high pressure liquid chromatography mass spectroscopy (LC-MS/MS) at The Hormone

Laboratory, Haukeland University Hospital [59]. The vitamin D-variable was available for adjustments in paper II and III.

3.4 Self-reported data

3.4.1 Assessment of puberty

Pubertal status in girls was determined through a question about age at menarche, for those who reported they had started menstruating. The Pubertal Development Scale (PDS) were introduced during the study [86], providing supplementary data on secondary sexual characteristics in boys. Sexual maturation was categorized into "Early", "Intermediate" and "Late" for girls, correspondingly "Completed", "Underway" and "Barely started" for boys, as described in paper I. In paper II and III the analyses were adjusted for sexual maturation as a continuous variable in girls and categorical in boys.

3.4.2 Assessment of physical activity

The participants responded to several questions on exercise and physical activity during leisure time. They were questioned about frequency, duration and intensity of the activities, as well as hours spent in sedentary activities during ordinary days.

Table 1 The Gothenburg Instrument

Question	**Answer options**
State your exercise and physical exertion in leisure time. If your activity varies much, for example between summer and winter, then give an average. The question refers only to the last twelve months.	1) Reading, watching TV, or other sedentary activity?
	2) Walking, cycling, or other forms of exercise at least 4 hours a week? (including walking or cycling to school, shopping, Sunday-walking, etc)
	3) Participation in recreational sports, heavy outdoor activities, snow clearing etc? (note: duration of activity at least 4 hours a week)
	4) Participation in hard training or sport competitions, regularly several times a week?

Table 1 shows a short instrument developed in Gothenburg in the 1960´s to measure leisure-time physical activity levels [87]. This instrument here denoted the Gothenburg

Instrument, is the main questionnaire used for assessing physical activity level in this thesis.

3.4.3 Assessment of other covariates

The *Fit Futures* survey included an extensive electronic questionnaire with numerous questions about lifestyle [88], and the experienced technicians interviewed the participants about past medical history and use of medication. The data management of the collected covariates are thoroughly described in the respective papers.

3.5 Statistics

Analyses were performed sex stratified using SPSS (Statistical Package of Social Sciences, Chicago, IL, USA) version 22. All tests were two-sided, and the level of significance was set to 0.05. Descriptive characteristics of the study population were presented as mean, SD and frequencies (%). Differences between groups were explored with Pearson´s Chi^2 test and "Independent samples *t*-test", while comparisons of the study population´s BMD-levels with the international references were performed with "One sample *t*-test for a single group" (Paper I). By Pearson´s R we explored correlations between variables. To assess differences between group means we used ANOVA, performed with the more conservative Bonferroni correction to reduce familywise error rate.

In paper I, we explored relationships by simple linear regression analyses between all relevant and available variables of importance for BMD-levels. Modelling to the highest adjusted R^2 by multiple linear regressions, we built a model explaining the variance in the study population the best way. In general, subjects with missing values in exposure, outcome or confounder variables were excluded form regression analyses. Due to the considerable missing of male puberty values, multiple imputations were performed [89]. Possible moderation effects between independent variables were checked and statistical significant interaction terms included in the models. When appropriate, stratified analyses to avoid the observed interaction effects were performed (Paper I and Paper III).

In paper III, we conducted ANCOVA and 2-ways ANCOVA to compare means across FM and LM-tertiles, and at different FM/LM-combinations, respectively. Carbonated

drink consumption, calcium intake and serum vitamin D-levels were not available for analyses in paper I. All these variables were included as confounding variables in paper II, and the two latter in paper III. In the third paper we estimated by logistic regression the impact of decreasing FM and LM on the probability of having low BMD-levels. After each model fit, residual analyses to assess assumptions of linearity, normality and homogeneity of variance, as well as multicollinarity were performed.

4. Results

4.1 Summary of papers

4.1.1: Paper I:

Hip fractures are regarded as the most serious osteoporotic fracture, and Norway has one of the highest reported incidences of hip fractures in the world. Maximization of peak bone mass may prevent later fractures. The aims of this population-based study were to describe BMD-levels in Norwegian adolescents, compare these levels with international reference ranges and explore associated factors that may influence peak bone mass acquisition at the femoral sites in this age group. The study cohort included 1,038 participants, while the main analyses comprised 469 girls and 492 boys younger than 18.

BMD-levels appeared higher in Norwegian adolescents compared to the Lunar pediatric reference data ($p<0.001$ at all sites for those 16 years old). In multivariate analyses height, participation in intensive physical activity of more than four hours a week and early sexual maturation were significantly associated with higher BMD levels at both femoral sites in girls ($p\leq0.019$). BMI was significantly associated with BMD only at the total hip ($p<0.001$). The corresponding variables in boys were age, height, BMI, higher physical activity levels and alcohol intake ($p\leq0.038$), whereas early stage of sexual maturity and smoking were negatively related to BMD ($p\leq0.047$). Snuffing and use of hormonal contraceptive had no significant influence on BMD levels. The highest physical activity levels were associated with more than 1 SD higher BMD levels than those reporting sedentary activities, which correspond to a fracture risk reduction of 50% in adulthood.

In conclusion, despite the heavy fracture burden, Norwegian adolescents´ BMD levels are similar or higher than age-matched Caucasian populations. Peak bone mass seems to be modifiable by lifestyle factors, such as physical activity, BMI, alcohol consumption and smoking. Physical activity were associated with considerably higher BMD levels at the hip in those involved in recreational sports or sports at a competitive level.

4.1.2: Paper II

Low levels of physical activity may have considerable negative effects on bone health in adolescence. The population´s opportunity to be sedentary, rather than active has increased, and self-reported media use appears to have increased in the past decades. In this study we explored the associations between self-reported hours spent in front of television/computers and BMD-levels measured in FF1. Persisting associations were explored by analyses of repeated bone measurements from FF2. Complete data sets for 388/312 girls and 359/231 boys at FF1/FF2 respectively, were used in analyses.

Boys spent more time in front of television and computers: mean 5.1 (SD 2.7) and 3.8 (2.2) hours per day in weekends and weekdays, respectively, compared with 4.0 (2.3) and 3.2 (2.0) hours in girls ($p<0.001$). Physical activity levels were inversely related to leisure time computer use at weekends ($p<0.001$). However, many adolescents balanced 2-4 h screen time with moderate or high physical activity levels. In FF1, screen time at weekends was negatively associated with BMD-levels in boys and positively in girls, after adjustments of age, puberty, height, BMI, physical activity, vitamin D levels, smoking, alcohol, calcium and carbonated drink consumption. This contrasting pattern was also observed in 2 years follow-up analyses.

This study suggests that for young boys, self-reported time spent on screen-based sedentary activity was negatively associated with BMD-levels; this relationship persisted 2 years later. Such negative associations were not present among girls.

4.1.3 Paper III:

BMI is positively associated with higher BMD-levels at all ages. In this study we examined the influence of body composition, in terms of total body lean mass (LM) and total body fat mass (FM) on BMD-values at the hip in 395 girls and 363 boys younger than 18. We also explored if certain combinations of the two components were more beneficial for bone health than others.

In analyses stratified for body composition, femoral neck BMD increased by 0.053 and 0.032 g/cm^2 per SD increase in LM and FM respectively ($p<0.001$) in girls, after adjustments for age, height, sexual maturation, physical activity levels, vitamin D levels, calcium intake, alcohol consumption and smoking habits. In boys, corresponding values were 0.072 and 0.025 g/cm^2, ($p<0.001$). When comparing means, the LM/FM combinations including high LM stood out as most beneficial for BMD. Decreasing LM was associated with an increased risk of osteopenia; with a doubled effect in boys, compared to girls.

Taken together, we concluded with a gender specific variation in LM´ and FM´ relationships with BMD. High LM was of crucial importance, observed more prominently in boys, while a more complex balance between LM, FM and BMD was observed in girls. In adolescents with lower LM, high FM seemed to offset the effect of deficient LM.

5. Discussion of methodological considerations

5.1 Study design

The cross sectional design can be used for studying diseases and risk factors in a populations in a narrow, defined period of time [90]. Its observational and descriptive nature makes it well suited to generate hypothesis. The study design is in general exposed to selection bias such as underrepresentation of diseases of high mortality (survivor bias), non-responder bias, as well as inter-observer bias [90]. The design´s main constrain is however, the limitation of causal inferences, as exposures and outcomes are collected at the same time. Nevertheless, strong associations, as observed in this study, may indicate true relationships.

5.2 Random error and precision

"Precision in measurement and estimation corresponds to the reduction of random error" [91]. Detailed protocols were developed by the Fit Futures administration before study start to prevent and minimize random and systematic errors. Likewise, efforts were put to monitor and maintain the quality of the data during the conduct of the study. The study was carried out in a specialized hospital ward, set up for medical research. The research technicians were trained in all data collection procedures, to reduce inter-observer variability. Anyhow, most variables are measured with some degree of error, variability we cannot predict [91]. These random errors have no preferred direction; some estimates will be too high and others too low. Larger sample size tends to eliminate the effect of random error and produce a correct estimate of the mean [92]. With a sample size of approximately 400 of each sex, random error and subsequently precision was likely not a problem, and the observed associations not a result by chance.

5.3 Systematic error and internal validity

5.3.1 Selection bias

Selection bias is a systematic error in a study related to procedures for selection of subjects, or from factors that influence study participation [91], and occur when the relationships between the associated factors are different in responders and non-responders. In Norway, all adolescents are enrolled in the upper-secondary school

system, ensuring complete registration of all students in this specific age group. Of the registered students, 70 quitted schools before study start, 114 did not attend school due to persistent diseases or we were unable to get in touch with them, and another 7% of the invited did not participate in the study (Fig 1, paper I). How non-attendance influences the results, will vary according to variables of interest. Dropout from school may be associated with an unhealthy lifestyle, and individuals suffering chronic illness may also have lower BMD levels [66], which could have caused an over-estimation of the study population´s BMD-levels. In contrast, comparisons of participants and non-attenders in FF2 revealed higher BMI-values among the non-attending girls (paper II). A corresponding bias in FF1 may have under-estimated the observed BMD-levels, and made these results more conservative. Thus, with data from 80% of the background population, which are considerable compared to other cross sectional studies, we believe that the results should not be twisted by selection bias.

5.3.2 Information bias

Information bias refers to a distortion of an estimate that occurs when measurement of either the exposure or the response is systematically inaccurate, and leads to different quality of information between compared groups [93]. Common reasons are imperfect definitions of study variables or incorrect procedures for data collection leading to misclassification (categorical variables) or measurement errors (continuous variables) [94]. Systematic errors may be non-differential or differential. The non-differential errors, i.e. when the error is independent of exposure and outcome, may mostly lead to attenuation of the result, whereas differential errors are more serious flaws. They occur when the error is *not* independent of exposure and outcome, and may twist the result in any direction, more difficult to predict, and must be handled in the planning process.

5.3.2.1 Validity of BMD-measurements

The DXA technique is regarded to have an excellent precision, despite some limitations related to soft tissue composition. In this study, we followed the calibrating procedures from the manufacturer, but did not perform a separate precision or accuracy study. However, the CV of our device has previously been estimated to 1.2 and 1.7% at the total

hip and femoral neck respectively [95], as reported by others [96]. However, the main outcome, BMD derived by DXA, may have been exposed to measurement errors. The DXA measurement is two-dimensional (2D), and does not take into account the third dimension - the size of the bone, and therefore unable to measure the true volumetric density [96]. As the depth of the bone is "hidden" in the 2D projected image, small bones are prone to under-estimation and larger to over-estimation of bone density. To handle this technical limitation, BMD-measurements in children and adolescents are usually reported in relation to age, gender and race. In addition, interpretation of the estimates should take into account puberty, as sexual maturity is closely related to height and growth [96]. By sex-stratified analyses, errors according to gender have been eliminated in this study. Extended analyses without the non-white participants (4%) did not change the results, indicating that influence of race was likely not an issue. All analyses were adjusted for age, height and puberty to minimize the effects of potential measurement errors.

5.3.2.2 Validity of puberty assessment

Tanner stages for puberty assessed by health professionals, have been regarded as the gold standard for classification of sexual maturity [97]. According to feasibility this approach was left out during the planning of the study. The included question on menarche age is well validated and provided us with reliable puberty data for girls [98]. During the survey self-rating questions on secondary sexual characteristics, the PDS were introduced, providing supplementary data on pubertal status in boys, too. The PDS-questionnaire administered by interview or by self-report has shown to be a reliable alternative to Tanner stages, although the validity has been questioned because of its broad estimates [86, 97, 99]. The questionnaire has been regarded appropriate for use in longitudinal studies, as well as in cross-sectional studies when rough estimates will be adequate [86, 97]. In this study, treated as a covariate we concluded the validity to be sufficient.

The late introduction of PDS resulted in considerable missing values, 23% in boys compared to 3% in girls. Questions on puberty may be a delicate subject and therefore inaccurate or not answered at all. However, it is most likely that such sensitiveness was

evenly distributed between the sexes. The proportion of unanswered puberty data in girls was in line with other lifestyle variables (2%), consequently we concluded with randomly missing puberty data in boys. This assumption was supported by sensitivity analyses discussed in paper I. Subsequently we performed the multivariate analyses including multiple imputations of missing values. It is likely that these pooled estimates explained the variability in the study population closer to the truth. Taken together, the potential errors related to puberty on the DXA measures must be considered controlled for, leaving us with robust estimates.

5.3.2.3 Validity of physical activity assessments

The physical activity data was collected by questionnaires, which implies some disadvantages. Misinterpretation of categories and memory problems may lead to misclassification. The Gothenburg Instrument used in previous Tromsø Studies correlates weakly but consistently with cardiorespiratory fitness of healthy adults in a Norwegian population [100]. Physical activity like running, jumping and ball games are related to aerobic capacity. As such activities also insert mechanical loading and muscle forces on the skeleton, we assume a similar relationship between the physical activity questionnaire and bone mass.

Over-reporting of physical performance is a matter of concern. However, the Gothenburg instrument showed a good relative ranking between participants in the Tromsø Study [101]. A comparison of this questionnaire and objectively measured physical activity levels by accelerometer were significantly correlated both for women and men [101]. The study concluded that despite a tendency of over-reporting by questionnaires, the adults estimated their physical activity levels in concordance with objectively measured levels. This questionnaire has yet not been validated for our age group. Social pressure may lead to over-reporting of physical activity among adolescent, too. However, such over-report will probably underestimate the observed association between physical activity and BMD levels.

The sedentary behaviour addressed as hours spent on television and computer use, must be considered as rough estimates (paper 2). This questionnaire did not elaborate sedentary activity in a broad matter. It did not distinguish between use of different

screen-modalities and no information about other sedentary activities was obtained. Social pressure may be an issue when it comes to screen time, under- as well as over-reporting is likely in this age group. Such misclassification in both directions may have resulted in an attenuation of the results. However, the consistency with previous findings in boys and persisting results over time indicates that the construct "screen time" captured a plausible association.

5.3.2.4 Validity of covariate assessments

The research technicians performed the data collection of height and weight, and the equipment was regularly calibrated. For these variables and BMI-values derived from these, measurement errors were of minor concern. According to vitamin D-levels, the blood-samples were analysed at a laboratory participating in the DEQAS quality programme, reporting a low CV [59]. Recall bias may have influenced all the self-reported data collection. For nutritional information, diseases, medication and contraceptives, this is most likely non-differential errors attenuating the results. However, it is well known that adult study participants tend to under-report smoking habits [102]. Likewise, such under-report may be an issue in adolescent too, also when it comes to alcohol consumption. In addition, these questionnaires were not validated for our age group. However, the results are in line with previous studies [41, 66], but must be interpreted carefully.

5.3.3 Confounding and interaction

To be considered a confounder, an extraneous factor needs to be associated with the exposure variable in question as well as the outcome, the BMD-levels. The confounding factor has to be causally related to the outcome, whereas the relationship to the independent variable may be of causal or non-causal nature. The confounded association is a real, but potentially misleading association [94]. If the influence of the confounder is unequally distributed between compared groups, it leads to invalid conclusions, unless it is planned for by randomization or matching, or handled in the statistical analyses [94]. In a cross-sectional study matching is not an option, so we used stratifications and adjustments strategies to reduce the influence of confounding factors. Identification of

potential confounding factors is based on knowledge in the field. Sex is regarded as a common confounder, and separate analyses were performed for girls and boys. Age and ethnicity likewise, however stratification may cause small subgroups, resulting in insufficient power to detect associations [90]. Thus, adjustments for age, BMI, height, sexual maturity, physical activity, alcohol consumption and smoking habits were included in all analyses, as these variables were considered to be the most important confounders. In paper III we stratified the analyses for fat mass and lean mass tertiles, not only to reduce the confounding effect but also to eliminate the observed interaction between the two variables. Interaction describes a situation in which two or more risk factors modify the effect of each other with regard to the occurrence or level of a given outcome [94]. However, unmeasured factors may have caused residual confounding. As discussed in paper II, other lifestyle variables or non-screen-based sedentary behaviour associated with screen time weekends and causal related to BMD, may have confounded the results.

In conclusion, a valid study or an "unbiased study" will on average, due to its design, methods and procedures, produce results that are close to the truth [94]. Based on the thorough planning, the high attendance rate, the appropriate measurements, questionnaires and analyses, we regard this study to be valid.

5.4 Generalizability - external validity of the results

Internal validity is a prerequisite for external validity, which "*is present when the results of a study are true and meaningful for a larger population and not only for the study participants"* [92]. Whether the results from this study are generalizable to other youth groups depend on the spectrum of individual characteristics in the study sample. If the distribution of characteristics is similar, the generalizability increases, which are also the case in studies with no self-selection or randomization, like the present study. The prevalence of overweight is higher among adolescents in the Northern parts of Norway compared to other parts of the country [103-105], and our youth group reported somewhat higher physical activity levels compared to 16-year-old participants in the Young-HUNT Study [106] (paper I). These important variables contribute to higher

levels of bone mass, consequently the reported BMD-levels may not be representative for all Norwegians in this age group. However, according to Rothman and Greenland scientific generalization in not simply a matter of statistical generalization. Abstractions from particular observations to universal statements are valid if the selection of study groups with its characteristics, distinguishes between competing scientific hypotheses in a proper way [91], as concluded by in the validity considerations.

The Tromsø study´s cohort consists of whole birth cohorts and random samples, and is regarded fairly representative for Caucasians in a national context [107]. Such an assumption is probably true for the younger *Fit Futures* cohort too. Due to the concordance to other findings, we believe the results from this study to be valid for Norwegian adolescents, and to some extend for Caucasians similar in age, living in Western countries.

6. Discussion of results

Through this study, we have learned that despite the heavy burden of fractures in elderly, Norwegian adolescents have similar or probably higher BMD-levels compared to age-matched European peers. Modifiable lifestyle factors like physical activity and BMI contribute considerably to BMD-levels in this age group, although the results are influenced by pubertal development. The observed trend of lower lean mass at declining physical activity levels attribute an adverse influence on bone health, especially among boys. While girls who compensate lower lean mass levels by fat mass, is better off with regard to bone mass.

6.1 Comparisons of BMD-values with international reference-values

The mean BMD-levels for age were not adjusted for height or weight, neither was the Lunar-reference. Differences caused by anthropometric measures were impossible to reveal, and the BMD-difference between the two groups may have been over-estimated because of a possible taller and heavier Fit Futures population, as discussed in paper I. Nevertheless, the consistency in these findings indicates that there is no reason to believe that Norwegian adolescents of today have lower BMD-levels than other white European youths. If this was the case for today´s elderly in their adolescence, we can however not know.

Until the mid-eighties measurements of height and weight was compulsory in all Norwegian schools, and the physical growth of schoolgirls in Oslo from 1918 to 1985 have been described [108]. Increasing overweight and obesity among children is worrying, and in 2008 the NIPH started the "Child Growth Study in Norway". Anthropometric measures in 8 years old children have been collected at approximately 130 schools across the country every second year since [104].

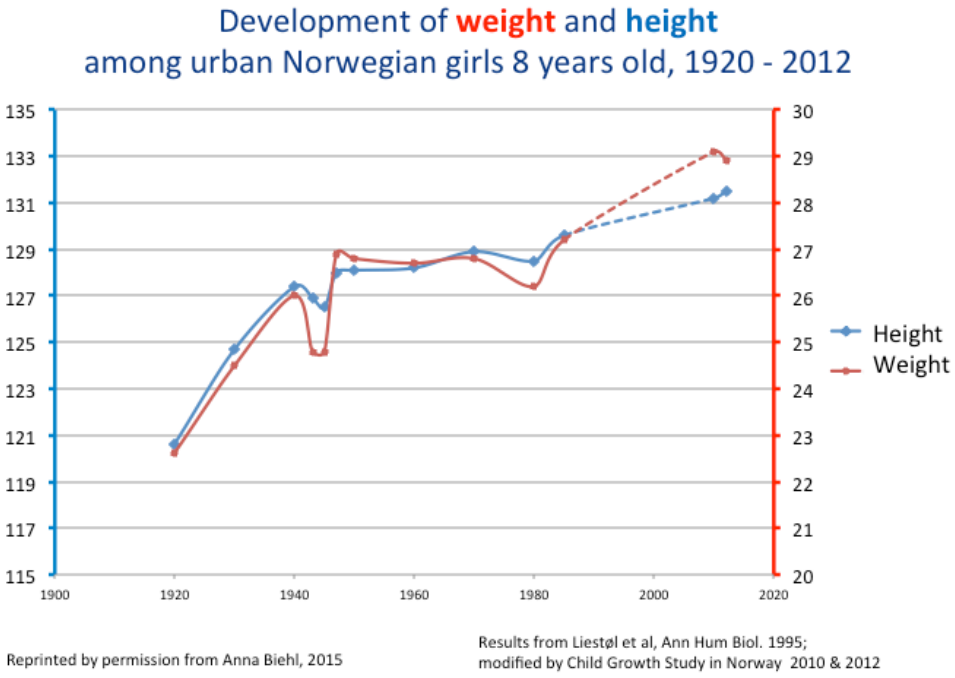

Figure 4 Height and height development in urban Norwegian girls.

By placing these results together, illustrated for urban girls in Figure 4, we see increasing height and weight during the 20th century and into the 21th, except during the 2nd World War. Increasing height and weight is closely related to nutritional state, and may have promoted higher BMD-levels among adolescents today, regarded as a cohort effect linked to better living conditions. The decreasing fracture rates observed in Norway [2, 16], supports such a conclusion. If the nutritional state in Norway and the Nordic countries were poorer than other parts of Europe during the last century, this may partly explain the high Nordic fracture rates among the elderly. It is possible that our findings announce that we will be observing future Norwegian cohorts with better bone strength and reduced fracture risk.

6.2 The physical activity – BMD relationship

In our cohort the physical activity levels were differently distributed between the sexes. The major part of the girls reported that they participated in recreational sports and moderate activities, whereas there was a polarized pattern among boys. Approximately

30% of the boys described their leisure time spent on sedentary activities and one of five participated in hard training and sports at a competitive level. We concluded in paper I that physical activity levels were higher in our cohort than in Young-HUNT. The eight FF1 schools included one school for athletes at a national level (Norges Toppidrettsgymnas) and some other schools offered education in dance and sports. It is likely that students at these "osteogenic" educations also reported higher levels of leisure time physical activity, and that their BMD-levels were attributed to an excess mechanical loading during the day. These observed effects are real, but may have over-estimated the observed BMD-increments at higher physical activity levels compared to the sedentary level. Objective measures of physical activity during the day would possibly shed light on this issue; however, the significance of higher physical activity levels for bone strength is in line with others [41, 64, 109, 110].

A recent study of bone strength and total volumetric BMD obtained by HR-pQCT in elite alpine skiers compared to recreational active students concluded that the higher bone strength achieved by micro-architectural adaptions was not apparent through the BMD measurements [111]. If this result also applies for areal BMD at a population level, the observed BMD-levels ´ significance for bone strength increases. Nevertheless, even if the influence of physical activity has been over-estimated, there is no doubt that low physical performance must be regarded a relative disadvantage for bone mass.

6.3 The body composition – BMD relationship

In adults body weight and BMI has been regarded as important for bone mass, and according to our results, this applies for adolescent bone too. Although a study population heavier than the general adolescent population in Norway may have attributed to the observed high BMD levels, the strength in the associations and the concordance with other results are convincing [46]. A decomposition of body weight into LM and FM may be suitable for a better interpretation of these relationships. Longitudinal studies of structural bone strength support LM´s and FM´s influence on BMD observed in this study [112, 113]. The described gender variation of different LM/FM combinations and BMD has not previously been elaborated in a longitudinal perspective, although Kouda et

al. from a cross-sectional study reported similar associations between FM and BMD for adolescents in lower LM-groups [114].

It is noteworthy that decreasing LM doubles the likelihood for lower BMD-levels, in boys compared to girls. That low physical performance in boys may be deleterious if they proceed that way, while girls to some extend will profit on increased FM related to sedentary behaviour, is a plausible inference. However, this is beyond the scope of the study´s cross-sectional design. Keeping the last century´s height and weight development in mind, combined with the decline in manual work because of mechanization and automation, the described decrease in fracture incidence, observed higher for women than men, may possibly be attributed to such a gender difference.

6.4 Other lifestyle factors and BMD

In paper I all available lifestyle factors´ relationship to BMD was explored. In girls and boys snuffing was not significant related to BMD ($p \leq 0.10$), neither was comorbidity or daily use of medication, and these factors were excluded from further analyses. Smoking was related to BMD in both sexes, whereas alcohol consumption only in boys. After multiple adjustments, the relationship between smoking and BMD attenuated in girls. In boys smoking was negative, whereas moderate alcohol consumption was positive associated with BMD levels, in concordance with previous studies [38, 66, 70].

The relationship of CHC use in girls was negative for total hip as describe by others [72, 73] and positive at the femoral neck, although not statistical significant. Twenty-two of the girls reported use of contraceptives containing progestin-only methods, of these only 10 reported injections or sub-dermal implants. In this study no consistent relationship of hormonal contraceptives and BMD levels were observed, despite injections of depot medroxyprogesterone acetate (DMPA), have been negatively related to BMD during such treatment [75, 115].

The cross-sectional design, providing only associations is the main limitation of this study. DXA-measures as a surrogate for bone strength are widely acknowledged,

however assessing bone strength in terms of micro-architectural adoptions, would have provided valuable insights. In addition, inclusion of socioeconomic adjustments, and particular issues as discussed in relation to each paper, would have strengthened the results.

7. Concluding remarks and further perspectives

This cross-sectional study describes the association of common lifestyle factors on bone mass in an age group just before bone mass peaks. Physical activity and weight, illustrated by body composition in terms of lean mass and fat mass, seem to be the most prominent predictors. Nutritional state is a minor issue for bone mass for the vast majority of Norwegian youths. However, there might be vulnerable groups due to illness or a combination of several adverse lifestyle factors. Our results suggest that in boys, lifestyle factors explain more of the variance in BMD-levels, compared to girls. Relatively sedentary boys, with excess screen time or boys with lower lean mass levels, had lower BMD levels, whereas sedentary girls were protected by their higher habitual fat mass. In this respect and given the remarkable changes in lifestyle compared to the last generations, male future bone health is worrying.

Weight, physical activity and tobacco use are modifiable. A follow-up study, with its stronger design provides an opportunity to confirm or set aside the associations observed in this cross-sectional study. In addition, objective measures of physical activity including frequency, intensity, type and duration should be included. Questions about the continuous effects of lower physical activity levels, the consequence of low adolescent BMI and LM in early adulthood, and if the longitudinal effects of snuffing are different from those of smoking, are relevant. Studies addressing the lifestyle variables´ persisting effect on bone mass from adolescence into early adulthood, just after the peak bone mass has been established, will generate new knowledge, as few longitudinal studies have examined this particular age span. Such information may contribute to knowledge-based preventive strategies of osteoporosis and fractures in a lifetime perspective.

References

1. *Osteoporosis and fractures in Norway - fact sheet*. Available from: http://www.fhi.no/artikler/?id=74450. Access date: 2015 May 26th
2. Omsland, T.K., et al., *Hip fractures in Norway 1999-2008: time trends in total incidence and second hip fracture rates: a NOREPOS study.* Eur J Epidemiol, 2012. **27**(10): p. 807-14.
3. *Consensus development conference: diagnosis, prophylaxis, and treatment of osteoporosis.* Am J Med, 1993. **94**(6): p. 646-50.
4. Hernlund, E., et al., *Osteoporosis in the European Union: medical management, epidemiology and economic burden. A report prepared in collaboration with the International Osteoporosis Foundation (IOF) and the European Federation of Pharmaceutical Industry Associations (EFPIA).* Arch Osteoporos, 2013. **8**(1-2): p. 136.
5. Borgstrom, F., et al., *The societal burden of osteoporosis in Sweden.* Bone, 2007. **40**(6): p. 1602-9.
6. Hansen, L., et al., *A health economic analysis of osteoporotic fractures: who carries the burden?* Arch Osteoporos, 2013. **8**(1-2): p. 126.
7. Dahl, C., *Quality of municipal drinking water and the risk of osteoporotic fractures in Norway*. 2014, University of Bergen.
8. Johnell, O. and J.A. Kanis, *An estimate of the worldwide prevalence and disability associated with osteoporotic fractures.* Osteoporos Int, 2006. **17**(12): p. 1726-33.
9. Cummings, S.R. and L.J. Melton, *Epidemiology and outcomes of osteoporotic fractures.* Lancet, 2002. **359**(9319): p. 1761-7.
10. Omsland, T.K., et al., *Mortality following the first hip fracture in Norwegian women and men (1999-2008). A NOREPOS study.* Bone, 2014. **63**: p. 81-6.
11. Osnes, E.K., et al., *Consequences of hip fracture on activities of daily life and residential needs.* Osteoporos Int, 2004. **15**(7): p. 567-74.
12. Johnell, O. and J. Kanis, *Epidemiology of osteoporotic fractures.* Osteoporos Int, 2005. **16 Suppl 2**: p. S3-7.
13. Cooper, C., et al., *Secular trends in the incidence of hip and other osteoporotic fractures.* Osteoporos Int, 2011. **22**(5): p. 1277-88.
14. Kanis, J.A., et al., *A systematic review of hip fracture incidence and probability of fracture worldwide.* Osteoporos Int, 2012. **23**(9): p. 2239-56.
15. Lofthus, C.M., et al., *Epidemiology of hip fractures in Oslo, Norway.* Bone, 2001. **29**(5): p. 413-8.
16. Emaus, N., et al., *Hip fractures in a city in Northern Norway over 15 years: time trends, seasonal variation and mortality : the Harstad Injury Prevention Study.* Osteoporos Int, 2011. **22**(10): p. 2603-10.
17. *Population projections, 2014-2100*. Available from: https://www.ssb.no/en/befolkning/statistikker/folkfram. Access date: 2015 May 26th
18. Omsland, T.K. and J.H. Magnus, *Forecasting the burden of future postmenopausal hip fractures.* Osteoporos Int, 2014. **25**(10): p. 2493-6.

19. Tortora, G.J. and B. Derrickson, *The skeletal system*, in *Essentials of Anatomy and Physiology*. 2013, John Wiley & Sons: Hoboken, NJ. p. 124-137.
20. Clarke, B., *Normal bone anatomy and physiology.* Clin J Am Soc Nephrol, 2008. **3 Suppl 3**: p. S131-9.
21. *Understanding bone Metabolism*. 2015; Available from: https://www.intmedpress.com/osteoporosis-online/?id=1969af4a-b5f5-49a9-b54d-3051eafdca0c&icid=6. Access date: 2015 September 7th
22. Bonewald, L.F., *Osteocytes*, in *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism*, C.J. Rosen, Editor. 2013, John Wiley & Sons. p. 34-41.
23. Ross, F.P., *Osteoclast Biology and Bone Resorption*, in *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism*, C.J. Rosen, Editor. 2013, John Wiley & sons. p. 25-33.
24. Yang, T., et al., *Human Fetal and Neonatal Bone Development*, in *Primer on the Metabolic Bone diseases and disorders of Mineral Metabolism*, C.J. Rosen, Editor. 2013, John Wiley & Sons. p. 121-126.
25. Wang, Q. and E. Seeman, *Skeletal Growth and Peak Bone Strength*, in *Primer of the Metabolic Bone Diseases and Disorders of Mineral Metabolism*, C.J. Rosen, Editor. 2013, John Wiley & Sons. p. 127-134.
26. Manolagas, S.C., M. Almeida, and R.L. Jilka, *Gonadal Steroids*, in *Primer of the Metabolic Bone Diseases and Disorders of Mineral Metabolism*, C.J. Rosen, Editor. 2013, John Wiley & Sons. p. 195-207.
27. Cooper, C., et al., *Epidemiology of childhood fractures in Britain: a study using the general practice research database.* J Bone Miner Res, 2004. **19**(12): p. 1976-81.
28. Bouxsein, M.L. and D. Karasik, *Bone geometry and skeletal fragility.* Curr Osteoporos Rep, 2006. **4**(2): p. 49-56.
29. Seeman, E. and P.D. Delmas, *Bone quality--the material and structural basis of bone strength and fragility.* N Engl J Med, 2006. **354**(21): p. 2250-61.
30. Seeman, E., *Structural basis of growth-related gain and age-related loss of bone strength.* Rheumatology (Oxford), 2008. **47 Suppl 4**: p. iv2-8.
31. Ammann, P. and R. Rizzoli, *Bone strength and its determinants.* Osteoporos Int, 2003. **14 Suppl 3**: p. S13-8.
32. Forwood, M.R., *Growing a Healthy Skeleton: The Importance of Mechanical Loading*, in *Primer of the Metabolic Bone Diseases and Disorders of Mineral Metabolism*, C.J. Rosen, Editor. 2013, John wiley& Sons. p. 149-155.
33. Bouxsein, M.L., *Technology insight: noninvasive assessment of bone strength in osteoporosis.* Nat Clin Pract Rheumatol, 2008. **4**(6): p. 310-8.
34. Johnell, O., et al., *Predictive value of BMD for hip and other fractures.* J Bone Miner Res, 2005. **20**(7): p. 1185-94.
35. Marshall, D., O. Johnell, and H. Wedel, *Meta-analysis of how well measures of bone mineral density predict occurrence of osteoporotic fractures.* BMJ, 1996. **312**(7041): p. 1254-9.
36. Cummings, S.R., D. Bates, and D.M. Black, *Clinical use of bone densitometry: scientific review.* JAMA, 2002. **288**(15): p. 1889-97.

37. Bachrach, L.K. and I.N. Sills, *Clinical report-bone densitometry in children and adolescents.* Pediatrics, 2011. **127**(1): p. 189-94.
38. Perez-Lopez, F.R., P. Chedraui, and J.L. Cuadros-Lopez, *Bone mass gain during puberty and adolescence: deconstructing gender characteristics.* Curr Med Chem, 2010. **17**(5): p. 453-66.
39. Baxter-Jones, A.D., et al., *Bone mineral accrual from 8 to 30 years of age: an estimation of peak bone mass.* J Bone Miner Res, 2011. **26**(8): p. 1729-39.
40. Bonjour, J.P., et al., *Peak bone mass.* Osteoporos Int, 1994. **4 Suppl 1**: p. 7-13.
41. Heaney, R.P., et al., *Peak bone mass.* Osteoporos Int, 2000. **11**(12): p. 985-1009.
42. Davies, J.H., B.A. Evans, and J.W. Gregory, *Bone mass acquisition in healthy children.* Arch Dis Child, 2005. **90**(4): p. 373-8.
43. Kanis, J.A., et al., *Assessment of fracture risk.* Osteoporos Int, 2005. **16**(6): p. 581-9.
44. De Laet, C., et al., *Body mass index as a predictor of fracture risk: a meta-analysis.* Osteoporos Int, 2005. **16**(11): p. 1330-8.
45. Lee, S.H., et al., *Bone mineral density of proximal femur and spine in Korean children between 2 and 18 years of age.* J Bone Miner Metab, 2007. **25**(6): p. 423-30.
46. Krahenbuhl, T., et al., *[Factors that influence bone mass of healthy children and adolescents measured by quantitative ultrasound at the hand phalanges: a systematic review].* Rev Paul Pediatr, 2014. **32**(3): p. 266-72.
47. Reid, I.R., *Fat and bone.* Arch Biochem Biophys, 2010. **503**(1): p. 20-7.
48. Travison, T.G., et al., *The relationship between body composition and bone mineral content: threshold effects in a racially and ethnically diverse group of men.* Osteoporos Int, 2008. **19**(1): p. 29-38.
49. Zhu, K., et al., *Associations between body mass index, lean and fat body mass and bone mineral density in middle-aged Australians: The Busselton Healthy Ageing Study.* Bone, 2015. **74**: p. 146-52.
50. Reid, I.R., *Relationships between fat and bone.* Osteoporos Int, 2008. **19**(5): p. 595-606.
51. Pedersen, B.K., *Muscle as a secretory organ.* Compr Physiol, 2013. **3**(3): p. 1337-62.
52. Yavropoulou, M.P. and J.G. Yovos, *Incretins and bone: evolving concepts in nutrient-dependent regulation of bone turnover.* Hormones (Athens), 2013. **12**(2): p. 214-23.
53. Ebbeling, C.B., D.B. Pawlak, and D.S. Ludwig, *Childhood obesity: public-health crisis, common sense cure.* Lancet, 2002. **360**(9331): p. 473-82.
54. Olds, T., et al., *Evidence that the prevalence of childhood overweight is plateauing: data from nine countries.* Int J Pediatr Obes, 2011. **6**(5-6): p. 342-60.
55. Weaver, C.M. and R.P. Heaney, *Nutrition and Osteoporosis*, in *Primer of the Metabolic Bone Diseases and Disorders of Mineral Metabolism*, C.J. Rosen, Editor. 2013, John Wiley & Sons. p. 361-366.
56. Matkovic, V. and R.P. Heaney, *Calcium balance during human growth: evidence for threshold behavior.* Am J Clin Nutr, 1992. **55**(5): p. 992-6.

57. Engelsen, O., et al., *Daily duration of vitamin D synthesis in human skin with relation to latitude, total ozone, altitude, ground cover, aerosols and cloud thickness.* Photochem Photobiol, 2005. **81**(6): p. 1287-90.
58. Winzenberg, T. and G. Jones, *Calcium and Other Nutrients During Growth*, in *Primer of the Metabolic Bone Diseases and Disorders of Mineral Metabolism*, C.J. Rosen, Editor. 2013, John Wiley & Sons. p. 142-148.
59. Oberg, J., et al., *Vitamin D deficiency and lifestyle risk factors in a Norwegian adolescent population.* Scand J Public Health, 2014.
60. Daly, R.M., *The effect of exercise on bone mass and structural geometry during growth.* Med Sport Sci, 2007. **51**: p. 33-49.
61. Greene, D.A. and G.A. Naughton, *Adaptive skeletal responses to mechanical loading during adolescence.* Sports Med, 2006. **36**(9): p. 723-32.
62. Frost, H.M. and E. Schonau, *The "muscle-bone unit" in children and adolescents: a 2000 overview.* J Pediatr Endocrinol Metab, 2000. **13**(6): p. 571-90.
63. Schoenau, E. and H.M. Frost, *The "muscle-bone unit" in children and adolescents.* Calcif Tissue Int, 2002. **70**(5): p. 405-7.
64. Bielemann, R.M., J. Martinez-Mesa, and D.P. Gigante, *Physical activity during life course and bone mass: a systematic review of methods and findings from cohort studies with young adults.* BMC Musculoskelet Disord, 2013. **14**: p. 77.
65. Warden, S.J., et al., *Physical activity when young provides lifelong benefits to cortical bone size and strength in men.* Proc Natl Acad Sci U S A, 2014. **111**(14): p. 5337-42.
66. Dorn, L.D., et al., *Longitudinal impact of substance use and depressive symptoms on bone accrual among girls aged 11-19 years.* J Adolesc Health, 2013. **52**(4): p. 393-9.
67. Yoon, V., N.M. Maalouf, and K. Sakhaee, *The effects of smoking on bone metabolism.* Osteoporos Int, 2012. **23**(8): p. 2081-92.
68. *Norhealth, Fact sheets*. Available from: http://www.norgeshelsa.no/norgeshelsa/?language=en. Access date: 2015 September 8th
69. Quandt, S.A., et al., *Smokeless tobacco use accelerates age-related loss of bone mineral density among older women in a multi-ethnic rural community.* J Cross Cult Gerontol, 2005. **20**(2): p. 109-25.
70. Berg, K.M., et al., *Association between alcohol consumption and both osteoporotic fracture and bone density.* Am J Med, 2008. **121**(5): p. 406-18.
71. Watts, N.B., *Estrogens, Estrogen Agonists/Antagonists and Calcitonin*, in *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism*, C.J. Rosen, Editor. 2013, John Wiley & Sons, Inc. p. 408-411.
72. Nappi, C., et al., *Hormonal contraception and bone metabolism: a systematic review.* Contraception, 2012. **86**(6): p. 606-21.
73. Jackowski, S.A., et al., *The associations of exposure to combined hormonal contraceptive use on bone mineral content and areal bone mineral density accrual from adolescence to young adulthood: A longitudinal study.* Bone Reports, 2015.
74. Tremollieres, F., *Impact of oral contraceptive on bone metabolism.* Best Pract Res Clin Endocrinol Metab, 2013. **27**(1): p. 47-53.

75. Isley, M.M. and A.M. Kaunitz, *Update on hormonal contraception and bone density.* Rev Endocr Metab Disord, 2011. **12**(2): p. 93-106.
76. Hough, S., *Fast and slow bone losers. Relevance to the management of osteoporosis.* Drugs Aging, 1998. **12 Suppl 1**: p. 1-7.
77. Rizzoli, R., et al., *Maximizing bone mineral mass gain during growth for the prevention of fractures in the adolescents and the elderly.* Bone, 2010. **46**(2): p. 294-305.
78. *The Tromsø Study*. Available from: http://www.tromsostudy.com. Access date: 2015 July 24th
79. *Declaration of Helsinki*. 2008; Available from: http://www.wma.net/en/30publications/10policies/b3/index.html. Access date: 2015 September 8th
80. *Health Research Act*. 2008; Available from: http://www.lovdata.no/all/hl-20080620-044.html. Access date: 2015 September 8th
81. *Patients´ Rights Act*. 1999; Available from: http://www.lovdata.no/all/nl-19990702-063.html. Access date: 2015 September 8th
82. *Lunar enCore, Supplement til pediatrisk referansedata*. 1. revision ed. 2010-Nov: GE Healthcare.
83. Cole, T.J., et al., *Establishing a standard definition for child overweight and obesity worldwide: international survey.* BMJ, 2000. **320**(7244): p. 1240-3.
84. Cole, T.J., et al., *Body mass index cut offs to define thinness in children and adolescents: international survey.* BMJ, 2007. **335**(7612): p. 194.
85. World Health Organisation. *BMI-classes*. Available from: http://apps.who.int/bmi/index.jsp?introPage=intro_3.html. Access date: 2013 October 22nd
86. Petersen, A., et al., *A self-report measure of pubertal status: Reliability, validity, and initial norms.* Journal of Youth and Adolescence, 1988. **17**(2): p. 117-133.
87. Saltin, B. and G. Grimby, *Physiological analysis of middle-aged and old former athletes. Comparison with still active athletes of the same ages.* Circulation, 1968. **38**(6): p. 1104-15.
88. *Fit Futures 1 Questback*. 2010]; Available from: https://web.questback.com/isa/qbv.dll/ShowQuest?Preview=True&QuestID=4130270&sid=OQgdIDT3Li&print=1. Access date: 2015 September 8th
89. Sterne, J.A., et al., *Multiple imputation for missing data in epidemiological and clinical research: potential and pitfalls.* BMJ, 2009. **338**: p. b2393.
90. Bhopal, R.S., *Concepts of epidemiology : integrating the ideas, theories, principles and methods of epidemiology*. 2nd ed. 2008, Oxford: Oxford University Press. XXXVII, 417 s.
91. Rothman, K.J. and S. Greenland, *Precision and Validity in Epidemiologic Studies*, in *Modern Epidemiology*, K.J. Rothman and S. Greenland, Editors. 1998, Lippincott Williams & Wilkins: Philadelphia. p. 115-134.
92. Jekel, J.F., D.L. Katz, and J.G. Elmore, *Epidemiology, Biostatistics, and Preventive Medicine*. Second ed. 2001, Philadelphia, Pennsylvania: Harcourt Health Sciences.
93. Last, J.M., *A Dictionary of epidemiology*. 4th ed. 2001, Oxford: Oxford University Press.

94. Szklo, M. and F.J. Nieto, *Epidemiology / beyond the basics*. 3rd ed. 2014, Burlington, Mass.: Jones & Bartlett Learning. XIII, 515 s.
95. Omsland, T.K., et al., *In vivo and in vitro comparison of densitometers in the NOREPOS study.* J Clin Densitom, 2008. **11**(2): p. 276-82.
96. Blake, G., J.E. Adams, and N. Bishop, *DXA in Adults and Children*, in *Primer of the Metabolic Bone Diseases and Disorders of Mineral Metabolism*, C.J. Rosen, Editor. 2013, John Wiley & Sons. p. 251-263.
97. Coleman, L. and J. Coleman, *The measurement of puberty: a review.* J Adolesc, 2002. **25**(5): p. 535-50.
98. Koo, M.M. and T.E. Rohan, *Accuracy of short-term recall of age at menarche.* Ann Hum Biol, 1997. **24**(1): p. 61-4.
99. Brooks-Gunn, J., et al., *Validity of self-report measures of girls' pubertal status.* Child Dev, 1987. **58**(3): p. 829-41.
100. Graff-Iversen, S., et al., *Two short questionnaires on leisure-time physical activity compared with serum lipids, anthropometric measurements and aerobic power in a suburban population from Oslo, Norway.* Eur J Epidemiol, 2008. **23**(3): p. 167-74.
101. Emaus, A., et al., *Does a variation in self-reported physical activity reflect variation in objectively measured physical activity, resting heart rate, and physical fitness? Results from the Tromso study.* Scand J Public Health, 2010. **38**(5 Suppl): p. 105-18.
102. Klesges, R.C., M. Debon, and J.W. Ray, *Are self-reports of smoking rate biased? Evidence from the Second National Health and Nutrition Examination Survey.* J Clin Epidemiol, 1995. **48**(10): p. 1225-33.
103. Groholt, E.K., H. Stigum, and R. Nordhagen, *Overweight and obesity among adolescents in Norway: cultural and socio-economic differences.* J Public Health (Oxf), 2008. **30**(3): p. 258-65.
104. *Resultater fra Barnevekststudien 2008-2012*. Available from: http://www.fhi.no/artikler/?id=107263. Access date: 2015 September 2nd
105. *Overvekt og fedme - Folkehelserapporten 2014*. Available from: http://www.fhi.no/artikler/?id=110553. Access date: 2015 September 2nd
106. Logstein, B., A. Blekesaune, and R. Almas, *Physical activity among Norwegian adolescents--a multilevel analysis of how place of residence is associated with health behaviour: the Young-HUNT study.* Int J Equity Health, 2013. **12**: p. 56.
107. Jacobsen, B.K., et al., *Cohort profile: the Tromso Study.* Int J Epidemiol, 2012. **41**(4): p. 961-7.
108. Liestøl, K. and M. Rosenberg, *Height, weight and menarcheal age of shoolgirls in Oslo - an update.* Ann Hum Biol, 1995. **22**(3): p. 199-2005.
109. Detter, F., et al., *A 6-year exercise program improves skeletal traits without affecting fracture risk: a prospective controlled study in 2621 children.* J Bone Miner Res, 2014. **29**(6): p. 1325-36.
110. Nilsson, M., et al., *Exercise during growth and young adulthood is independently associated with cortical bone size and strength in old Swedish men.* J Bone Miner Res, 2014. **29**(8): p. 1795-804.
111. Liphardt, A.M., et al., *Bone micro-architecture of elite alpine skiers is not reflected by bone mineral density.* Osteoporos Int, 2015. **26**(9): p. 2309-17.

112. Streeter, A.J., et al., *Body fat in children does not adversely influence bone development: a 7-year longitudinal study (EarlyBird 18).* Pediatr Obes, 2013. **8**(6): p. 418-27.
113. Jackowski, S.A., et al., *Does lean tissue mass accrual during adolescence influence bone structural strength at the proximal femur in young adulthood?* Osteoporos Int, 2014. **25**(4): p. 1297-304.
114. Kouda, K., et al., *Fat mass is positively associated with bone mass in relatively thin adolescents: data from the Kitakata Kids Health Study.* Bone, 2014. **64**: p. 298-302.
115. Cromer, B.A., et al., *Bone mineral density in adolescent females using injectable or oral contraceptives: a 24-month prospective study.* Fertil Steril, 2008. **90**(6): p. 2060-7.

Appendix A

Pamphlet of information

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
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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.

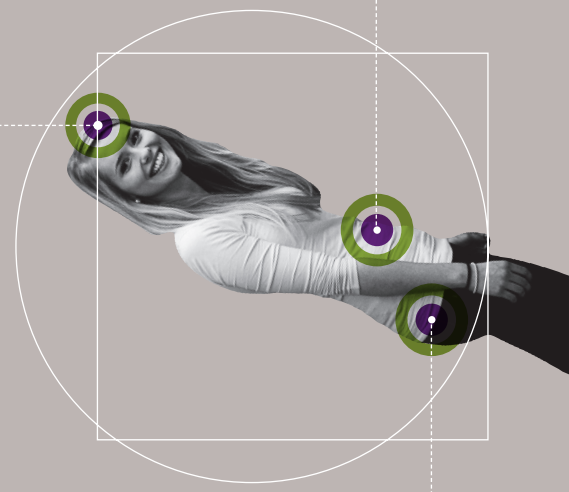
www.fitfutures.no

UNN – VI BRYR OSS – SAMMEN TAR VI ANSVAR

UNIVERSITETET I TROMSØ

folkehelseinstituttet

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 *smertefølsomhet* 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

TEKNOLOGI

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 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.

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.

SMERTE

MILJØGIFTER

RØYK
OG SNUS

Appendix B

Consent of participation *Fit Futures 1*

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)

Appendix C

Interview guide *Fit Futures 1*

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

Appendix D

Web link to questionnaires *Fit Futures 1*, 2010/2011

Fit Futures 1, 2010/2011
Questionnaires:

https://web.questback.com/isa/qbv.dll/ShowQuest?Preview=True&QuestID=4130270&sid=OQgdIDT3Li&print=1