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The association between sugar sweetened or carbonated beverages and bone mineral density in adolescents taking part in the Fit Futures Study.

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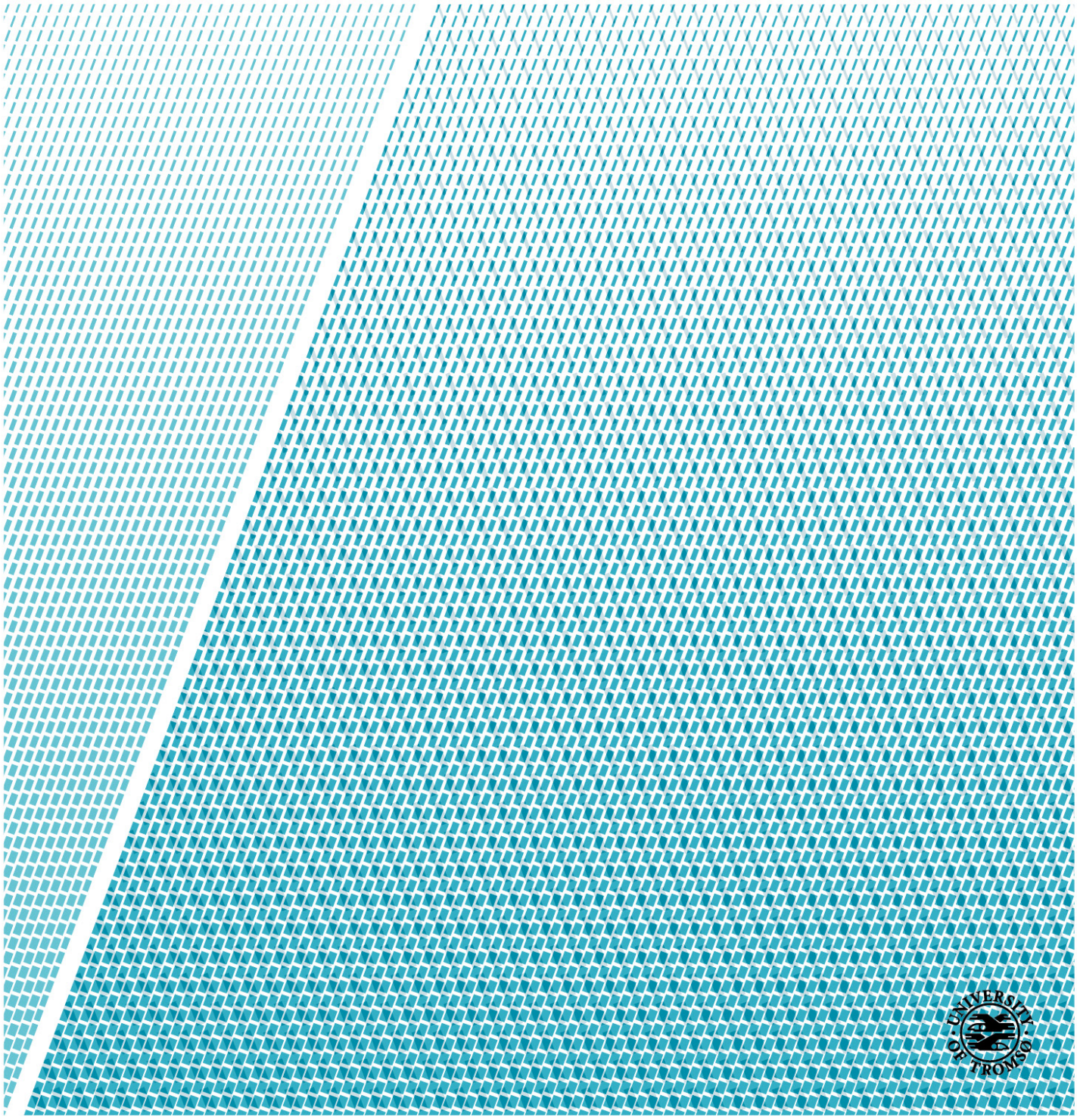

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Abstract

General info: In the prevention strategies for osteoporosis attention has been directed towards the acquisition of peak bone mass and early life experiences during the adolescent growth period. High consumption of soft drinks during adolescence is believed to influence bone mineral accrual and increase fracture risk later in life. The aim of this study is to investigate if moderate or high consumption of sugar sweetened beverages and carbonated beverages is associated with bone mineral density in adolescents taking part in the Fit Futures study.

Data and method: The thesis is a cross-sectional analysis from Fit Futures 1, a part of the Tromsø Study, including 1st year upper secondary school students. The study included 900 adolescents from the municipality of Tromsø and Balsfjord. Our main outcome, BMD of total body was measured as g/cm^2 by dual x-ray absorptiometry. Lifestyle and dietary variables were created from a self-reported questionnaire and interview. Descriptive and unadjusted analysis were first performed to explore the relationship between variables, and then a manual hierarchical block analysis was used to select the most influential predictors to BMD total body. In the main analysis, we used multiple regression to investigate the association between sugar sweetened or carbonated beverages and BMD. The multiple regression analysis was stratified by gender, and adjusted for; age, BMI, sexual maturity/puberty, main high school program, physical activity level, daily screen time, alcohol use, and artificial sweetened beverages.

Results: For the girls who reported to not drink any sugar sweetened beverages, there was found a significant association with higher BMD values (0.016 g/cm^2) compared to girls drinking half a glass daily. Other than that there was found no significant association between sugar sweetened beverages or carbonated beverages and BMD total body for both sexes when adjusted for the other confounding variables.

List of abbreviations

ATC - Anatomical Therapeutic Chemical Index

BMC - Bone mineral content

BMD - Bone mineral density

BMI - Body mass Index

CI - Confidence intervals

CS – Carbonated beverages

CV - Coefficient of variation

DXA - Dual-energy x-ray absorptiometry

FF - Fit Futures

FFQ - Food frequency questionnaire

FF1 - Fit Futures 1

LM - Lean mass

OC - Oral contraceptives

PBM - Peak bone mass

PDS - Pubertal development scale

PTH - Parathyroid Hormone

REK - Regional Committees for Medical and Health Research Ethics

ScT – Screen time

SD - Standard deviation

SPSS - Statistical Package of Social Sciences software

SSB – Sugar sweetened beverages

UiT - The Arctic University of Norway

UNN - University Hospital of North Norway

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1. Introduction

1.1 Background

Osteoporosis is a health issue that today still represents a major concern to public health. It is a disease that is characterized by increased bone fragility due to micro-architectural deterioration of bone tissue leading to high fracture risk. [1] The condition is a contributing factor to the majority of fractures in the elderly population. [2]

Norway has one of the highest incidences of hip fractures and wrist fractures worldwide [2]. Hip fractures are considered the most serious consequence of osteoporosis, and in Norway approximately 9000 new fractures are recorded every year. Since women are more prone to the condition due to lower bone mass and estrogen depletion in menopause, 7/10 hip fractures appear in women. [2]

Around 21 % of women and 33 % of men die within the first year after a hip fracture [3], and approximately 1/3 of all elderly who has encountered a hip fracture also become dependent on walking aids and further care in nursing homes after an incident. [2] In 2014 it was estimated that the annual expenses of hip fractures per year for the Norwegian society was around 7-9 billion kroners. This is without considering the additional costs created by institutionalized patients. [2]

Future predictions say that more than 41 million women all over the world will develop osteoporosis within the next 20 years if the situation remains unchanged [4]. However, in the recent decade a greater shift away from the perspective of treatment and more towards preventative measures has emerged. Gaining knowledge and understanding of which factors influence bone health has therefore received broader attention in research. [4]

1.2 Peak Bone Mass

During adolescence, the skeleton goes through a substantial and rapid growth spurt of bone accrual where the skeleton increase in thickness and become stronger. Although there is some dispute about the timing, there is a broad consensus that in the late teens or early adulthood this increase of bone mass reaches a plateau, referred to as Peak Bone Mass (PBM).[5] Lifestyle factors and early life experiences influences our physiology and the genetic constitution for bone mass acquisition. Sickness, unhealthy lifestyle, and unbalanced nutrition can therefore affect the growth of the skeleton and lead to various degrees of bone deposition. [5] In recent years much more attention has been directed towards the acquisition of PBM and early life experiences during the adolescent growth period in relation to optimizing good bone health later in life.[6]

In former research it has been reported that approximately 90 % of our bone mass is achieved by the age of 18 [7], and the amount of bone mineral you gain in these early adult years can explain as much as 60% of the risk of osteoporosis [6]. Former research has also suggested that one standard deviation higher bone mass at the end of skeletal maturity can reduce the future risk of fractures by as much as 50 %[7]. More knowledge about lifestyle factors and experiences that influence the genetic potential for gain in bone mass is therefore important in the prevention strategy for osteoporosis. [7]

1.3 Nutrition and Peak Bone Mass

Bone cells, like other cells, are reliant on nutrition to keep up the cellular processes of deposition, maintenance, and repair of bone tissue. Vitamin D, Calcium, and phosphorus are examples of some of these nutrients. Vitamin D is a key factor in stimulating bone health and an important moderator for the absorption and transport of calcium in the body. Vitamin D is produced in the skin from sunlight, but can also be consumed from sources in the diet, like for example fatty fish and cod liver oil. [8]

Calcium and phosphorus are minerals that are especially important for cellular activities, and our skeleton also functions as a large reserve for these two nutrients. Approximately 85 % phosphorus and 99 % calcium in the body is found in bones.[9] The size of our skeleton is linked to the size of this reserve, which is dependent on the intake from our diet and excretory loss.[5]

It is the parathyroid hormone (PTH) that is responsible for balancing the right levels of calcium and phosphorus in the blood. When calcium level is low, release of PTH will activate the kidney to produce more vitamin D, and this helps the body to take up more calcium and phosphorus from the intestines. The hormone also signals the release of calcium and phosphorus from bones into the bloodstream, and phosphorus excretion through the urine. [9] Phosphorus is not as strictly regulated as calcium, and with the consumption of high phosphorus food, the blood levels can elevate quickly. This can affect the absorption of calcium by altering the production of active vitamin D. Low levels of calcium in the blood leads to high stimulation of PTH, which can lead to resorption of bones. [10]

Numerous of studies have already demonstrated the importance of calcium for the maturation and preservation of bone integrity. [4] The absorption and utilization of calcium is a complex process that is determined by many aspects like physiological factors, medications, life stages, lifestyle, gender, and pathological conditions. A lot of the variability in calcium absorption is still not known and may be due to the variation of genetics [11].

Calcium is present in water and various types of food like for example cheese, milk, and yoghurt. Vegetables like kale, turnip greens and broccoli also contribute to the intake of dietary calcium. [8] Calcium can however only be absorbed in the intestines in its positively charged molecule form as Ca2+. In food, calcium is found as insoluble salts and is released when acidic pH is at a mild level. [4] This process is however more complex as calcium under

alkaline conditions also can combine with other minerals and constituents of the human diet, which can lead to restrictions in availability and absorption of the ingested calcium. The calcium status of the body also determines the efficiency of the absorption process. In periods of rapid growth, like pregnancy, childhood, and puberty in adolescence, up to 75 % of dietary calcium can be absorbed. [4] Since the body is not able to produce new calcium, a sufficient intake through our diet to preserve optimal bone health is important. [12] The recommended intake of calcium varies by age and gender, but for Norwegian adolescents that are 13-17 years old, the recommended intake is set to 900 mg/d. [13] Calcium is also a threshold nutrient, which means intake of the mineral after a certain threshold won't give further positive gains of bone. If the intake of calcium is beneath the sufficient level, the potential to reach the genetically predetermined peak bone mass could be altered. [5]

1.4 Sugar Sweetened and carbonated soft drinks beverages

Many factors are assumed to influence the optimization of bone accrual in the early decades of life. Nutrition, physiological factors, and the level of physical activity are well established to be important predictors. [14] In the recent decade, soft drinks containing caffeine, phosphorus, high content of sugar, and carbonation have received attention for its possible deleterious effects on bone and the calcium metabolism. [15]

A soft drink is defined as a cold non-alcoholic water based beverage. Usually the drinks are carbonated, flavored, and sweetened either natural (sugar content like fructose corn syrup, sucrose, fructose etc.) or artificially (sugar substitutes). The beverages usually also contain other ingredients like preservations, colorings, phosphoric acid, and sometimes caffeine. [16] [17] Exaggerated intake of some these substances found in soft drinks have been linked to bone resorption by altering the calcium homeostasis, or due to changes in the calcium-phosphorus ratio. [12] [18] This is especially of concern since the consumption of soda and sugar sweetened

beverages has increased among adolescents in the past decades. Statistics has documented that the annual turnover of carbonated beverages between 1950 and 2002 increased fifteen times in Norway.[19] After 2002 it receded, but compared to the rest of the world Norway is still a leading country in relation to soft drink consumption. Sugar sweetened soft drinks and lemonade are also responsible for 50 % of the total sugar consumption for Norwegian adolescents. [19]

In two cross sectional studies executed by Wyshak (1994, 2000) and his colleagues they found strong associations between carbonated beverage consumption and bone fractures in girls. High intake of dietary calcium on the other hand seemed to have a protective effect. [18,20] In another observational cross sectional study including 1335 boys and girls aged 12-15 years' old, they found associations between carbonated soft drink consumption and lower bone mineral density at the heal bone in girls [21]. High consumption of carbonated soft drinks has also been found to be significantly associated with hypocalcemia (low calcium levels in the blood serum) in case control studies in both children and adults [22,23].

There is an ongoing debate of whether the potential negative effects that soft drinks have on bone is due to the displacement of important sources of calcium in the diet, like dairy products or other mechanisms [24]. In a cohort study by Libuda et. al. (2008) they looked at the consumption of various types of soft drinks from 3-day dietary records (including caffeinated, uncaffeinated, carbonated, uncarbonated, sugar and artificially sweetened beverages) and possible association with bone variables of modeling and remodeling in 228 healthy children and adolescents. In the study they adjusted for covariates like age, sex, energy intake, muscle area, BMI, and growth velocity. They found that high intake of all soft drinks were negatively associated with lower bone mineral content (BMC), cortical area and polar strength strain index (surrogate measure of bone strength) for both genders. They also concluded that the

intake of long term protein effectuated the catabolic effect that soft drinks can have on BMC.[25]

Findings in other studies are also inconsistent. Some studies suggest that soft drinks only affect BMD and bone fractures in adolescent girls, not boys [20]. Others indicate that only soft drinks like Cola have a negative effect, due to mechanisms such as caffeine level, low ph-values, and the phosphoric acid. [26 27 28]

Exaggerated intake of sugar in relation to bone health has also been investigated. Studies have found that high intake of glucose can lead to excess of calcium in the urine, further leading to changes in the renal cell metabolism and affecting calcium reabsorption. [8] Others have also suggested that different mechanisms such as the insulin response to sugar can lead to inhibition of calcium reabsorption. [8]

A study was conducted on growing rats to see if feeding them various sugar sweetened beverages had an effect on bone mass and strength. The rats were fed either a dose of distilled water, or a solution containing glucose, sucrose, fructose, or high fructose corn syrup for 8 weeks. They found that the solution of glucose had the most negative effect in comparison to the fructose sweetened solution, which did not significantly differ from the control group. The researchers concluded that high intake of glucose led to excessive thirst, which again induced a significant decrease in mineral ingestion, resulting from lower consumption of food. This despite of an increased intake of calories. Calcium excretion was increased due to a low intake of phosphorus and calcium through the diet, and this led to a significantly lower BMC.[8]

1.5 Known determinants for peak bone mass

Proper nutrition, physiological factors, and physical activity are established predictors for optimization of bone accrual. Even though former research has suggested that 85 % of the observed differences in PBM is determined from genetic factors [14], it is through our lifestyle we are able to influence the acquisition of PBM. Especially since we are faced with an aging

population and dietary patterns are continuously shifting, it is important to know more about how our lifestyle can affect bone health.

1.5.1 Body Mass Index

BMI is considered to be of a positive influence on bone for both adolescents and adults, and approximately half of the bone mass variance is estimated to be explained by body weight. [29] Body weight/fat is considered to have a protective effect against future fractures due to the gravitational loading and mechanical stimulation of bone by additional weight. Many studies have confirmed this positive association, and also that active hormones from the fat tissue, muscle, and gut can exert an anabolic effect on bone mass. [29] On the other hand, there is also research that indicates that this positive effect by body fat only goes as far as to a certain limit. Some studies have indicated that lean mass is the driving force for maintaining good bone health. [30] [29] Whether the relationship between body composition and BMC is detrimental or protective is a complex issue and is still very much discussed in research.

1.5.2 Sexual Maturation

The accrual of bone mineral content during childhood and adolescence is dependent on many factors like body composition, sexual maturation, and growth. The start of puberty is related to peak bone mass with an inverse relationship, meaning that the adolescents who start puberty late also have lower BMC in their early adult years.[31] Height is also associated with bone accretion and a predictor for the size of the skeleton. Therefore, children who are considered tall in relation to other children their age have more substantial bone mass and density. However, the effect of height is different in relation to pubertal stages. The acquisition of bone mass in relation to puberty is a very complex relationship, but important

in understanding bone health in children and adolescents. [31]

1.5.3 Physical activity

The positive effect of physical activity on bone accrual has been established through numerous of studies [32] [21]. Physical activity stimulates osteogenic responses with mechanical loading that leads to alteration in bone structure and geometry, and thereby reducing bone loss. [12] It is well stated that physical activity has a positive effect on both BMC, BMD, bone size and strength [21]. By being active we also improve other aspects like flexibility, coordination, balance and endurance. [12]

There has been some dispute about what aspect of exercise is most effective for improving BMD in regards to intensity, duration, and frequency. In the systematic review by Bielemann et al. (2013) where they investigated the longitudinal association between physical activity and BMC for young adults, they found a consensus that sports with high intensity and high load on the skeleton gave improvements in PBM [33]. In another study from Fit Futures, Christoffersen and his colleagues (2015) wanted to know if frequency and intensity of physical activity had any impact on bone. They concluded that increased level of physical activity for adolescents was associated with elevated BMD and BMC levels. Activity with high frequency for both girls and boys had strong positive associations with BMD. Hard intensity activity also had an additional impact on BMD and BMC levels for boys. [32]

1.5.4. Screen-Time

The rapid technological development has raised concerns about children's and adolescent's sedentary behavior. We have seen an increasing trend of screen time, such as watching television and playing video games, replacing the time spent on sports and physical activity [34]. The Norwegian Directorate of Health monitor development in regards to public health. A

consistent finding in their reports is that in general the Norwegian population believe they are more active and less sedentary than they in reality are when measured by accelerometers or other objective methods. Reports show that the activity level is steadily decreasing from the age of 6 up to the age of 20. The activity level of a 9-year-old is estimated to be 40 % higher than that of a 15-year-old. Further it was observed a reduction in activity level of 31 % from the age of 15-20 years old. These numbers are based on recommendations of 60-minutes moderate activity daily. [35] Former studies have found a decrease in bone mass in relation to increasing screen time in adolescents [34].

1.5.5 Alcohol and Tobacco use

Alcohol has been found to have a negative effect on bone in many studies, and overconsumption has been related to low BMD in adults. [36] [29] There are on the other hand many studies that support the conclusion that moderate consumption of alcohol can have beneficial or protective effect on bone [29]. A study by Wosje and Kalkwarf (2007) investigated the relationship between intake of alcohol and bone mass in young adult men and women. They concluded that men who had been drinking more than five occasions/month had significantly higher BMD at the hip and femoral neck compared to men who drank less or abstained from alcohol. Consumption of alcohol had a positive effect on bone for men, but this effect was not found in men who were binge drinking. [37] Another study also found that moderate consumption of alcohol, meaning up 2 two drinks, had a positive protective effect on bone. [38]

There is still a lot of dispute about what effect alcohol have on bone, and the relationship to peak bone mass in young adults and adolescents is still not clear [39] [5]. More research is needed to establish the effect alcohol can have in relation to differences in age and sex [40].

Smoking has also been associated with lower BMD-levels. In a meta-analysis [41] that investigated cross sectional studies and prospective human studies, they collected studies that had quantitative measure of bone mass and pooled data across 86 studies with 40 753 participants in total. In the study they concluded that with a dose-dependent relationship, smokers at all bone sites had significantly reduced bone mass compared to never and former smokers. The effect was more substantial at the hip for men and in elderly. In the prospective studies, after controlling for age and weight, a larger bone loss was found over time in smokers compared to those who did not smoke, leading to increased fracture risk for both sexes. There were also indications that smoking cessation had a positive influence on bone mass. [41] There are some however some contradictive findings in other studies [5] [42], but these results may be due to differences in smoking habits and the capacity to control for confounding variables [29] .

There is still very limited research on the use of snuff and its possible effect on bone mass. In Norway snuffing habits has increased among adolescents since 2005, while the number of smokers has declined. [29] Since the mechanisms behind bone loss from smoking has not been clearly established, and the nicotine level in snuff is double the amount compared to cigarettes, is not unlikely to think that snuff could have negative impact on bone as well. [43]

1.5.6 Contraceptives, medication, and chronic diseases

The use of hormonal contraceptives has been related to bone health. [44] Oral contraceptives are used by millions of young women all around the world, and research have indicated that the use of this birth control can have different effects in young adult girls where peak bone mass is not yet fully developed compared to older women. In a cross sectional study [45] they studied both duration and the dosage of estrogen that 606 women aged 14-30 years took and its relationship to BMD. The researchers found that women aged 19-30 years had a lower

average BMD at the spine, whole body and hip with increased duration of OC. This result is conflicting with other studies that report no effect. The conflicting results most likely reflect the different hormonal composition in various types of oral contraceptives, and complexity of multiple estrogenic effects contraceptives can have on bone density. Prospective studies on adolescents are also still limited. [45]

Chronic illness with its associated comorbidities and side effects of different treatments are known to have negative effect on bone mass development in both adolescents and adults. [46] Chronic diseases that can lead to prolonged inflammation, malabsorption, reduced physical activity level, pancreatic insufficiency, or delay in puberty, can inhibit bone formation and maturation in different ways. [46]

Medications like for example glucocorticoids, oral corticosteroids are also well documented to have an cumulative effect on bone resorption and inducing osteoporosis and fracture risk in adults [47]. Studies on children and adolescents are still conflicting, especially in relation to the use of short courses of oral corticoids. There is also a lack of published prospective studies investigating the effect of inhaled corticosteroids in relation to f.ex treatment of asthma. [47]

1.5.7 Ethnicity

Difference in BMD and fracture risk for both sexes in different ethnic groups have been reported [48]. Higher fracture risk has been suggested in adult age-matched white American or British/European populations compared to other ethnic groups. Compared to the western population, greater incidences of hip fractures have also been reported in the Asian population [49] [50]. However, more data is still needed to confirm these results.

1.6 Aim of this study

Exaggerated intake of some substances found in soft drinks have been linked to influence BMD and bone health. In this study we wanted to investigate whether moderate or high consumption of sugar sweetened beverages and carbonated beverages potentially was associated with bone mineral density in adolescents taking part in the Fit Futures study – part of the Tromsø study.

2. Material and Method

2.1 The Fit Futures study

The data used in this cross-sectional study is provided from the Fit Futures study (FF).

FF is a comprehensive longitudinal youth survey and a supplement to the Norwegian Tromsø Study [51]. FF measures various indicators of lifestyle and health among young adolescents in the municipalities of Tromsø and Balsfjord (see appendix 1), and the survey is a collaborative project between the UiT the Artic University of Norway, The Norwegian institute of Public Health (NIPH), and the University Hospital of Northern Norway (UNN). [29] FF was implemented by the Department of Community Medicine at UiT the Artic University of Norway in 2010/11 (Fit Futures 1), and again in 2012/13 (Fit Futures 2).

In Fit Futures 1, first grade students from eight different upper secondary schools in Tromsø and Balsjord were invited to participate in the health survey. The study included a total of 1038 students (508 girls and 530 boys), providing an attendance rate of 93 %. [29] In 2012/2013 the survey was performed again (FF2) with repeated measurement of the participants in FF1,

and all new 3rd grade students attending the different schools. Because of higher attendance rate, and a larger sample, only data from FF1 will be used for analysis in this thesis.

The Tromsø Study was conducted for the first time in 1974 and has been performed in total 6 times.[51] The health survey collects data on various health problems like heart disease, lung diseases, diabetes, mental disorders, cancer, dementia, thyroid disorders, skeletal disorders and osteoporosis. The Fit Future study is intended to supply to the Tromsø Study with more research on the younger population. [52]

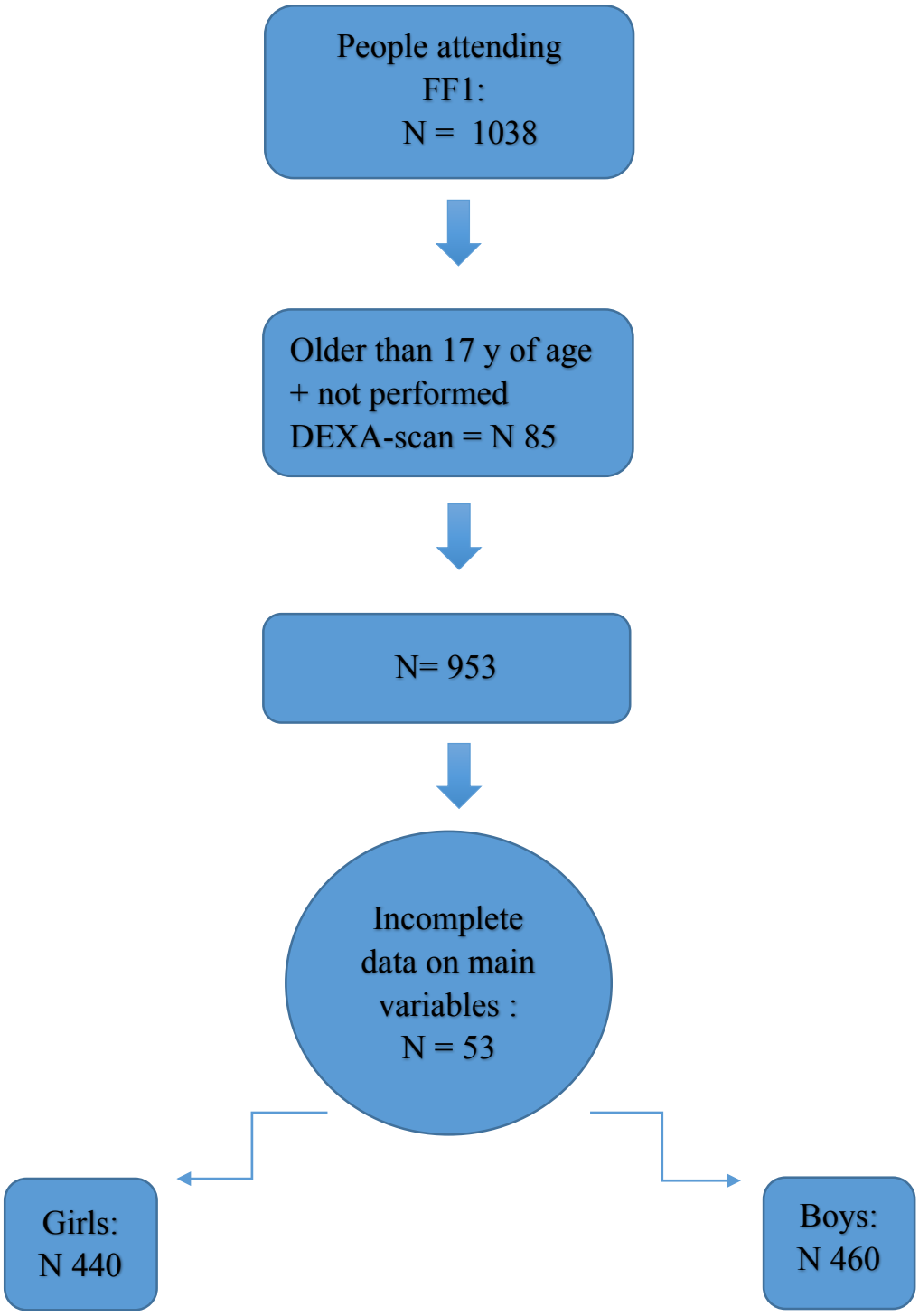
2.2 The study population

The eight schools which participated in FF1 offered a broad variation of academic disciplines, like general studies, several different vocational programs, and program for sports and physical education. 1301 students were registered to start first year of upper secondary school, but for various reasons some students did not start school, or had already quit school before the survey started. 1117 students were invited to take part in the cohort, and a total of 1038 students agreed to attend[29].

2.3 Inclusion and exclusion criteria

The participants in FF1 included adolescents from 15-28 years of age. Participants that were 18 years or older were excluded from this study due to the fact that Norwegian adolescents attend first year of high school when they are approximately 15-17 years of age when following a normal study course. Participants that for some reason had not attended a DXA-scan were also excluded. Persons with missing data on main variables were also eliminated for the reason that it was more valuable to have a similar and comparable group as a foundation in the analysis (see figure 1).

Figure 1. Flowchart of the thesis with BMD total body as outcome variable.



2.4 Data Collection

The survey was performed at UNNs clinical research unit and consisted of three parts where the students had to fill out a self-administered questionnaire, go through a clinical interview, and then take part in a general health examination. The interviews and anthropometrical measurements were executed by experienced and trained personnel. [29]

The self-administered questionnaire (see appendix 3) included general questions on lifestyle and nutrition, general wellbeing, diseases and health problems, personality, and family relations. The data program" Questback" was used for this questionnaire.

Questions about use of medication, chronic diseases and sexual maturity were some of the additional data collected in the interview. Information regarding the students' academic discipline was gathered from the school records. [29]

In the physical examination, a body scan of body composition was performed using a dual-energy x-ray absorptiometry (DXA). DXA is a low dose x-ray examination used to measure bone loss and bone mineral density, and is a commonly used method to diagnose osteoporosis. The procedure is performed with the participants laying still in an x-ray machine for approximately 10 minutes while their body is being scanned (see Appendix 1). All scans were performed in relation to the manufactured protocol by specially trained technicians at the hospital. Measurement of height and weight were also taken of all the participants.

2.5 Ethical considerations and consents

Fit Futures was approved as a licensed extension to the Tromsø Study by the Norwegian Data Protection Authorities. In 2009 FF was approved by the Regional Committee for Medical and Health Research Ethics (REK) with reference nr. 2009/1282. [29]

This master thesis project was also approved by REK with reference number 2016/2050.

All subjects participating in FF1 were provided with a descriptive information leaflet regarding the survey (Appendix 1). An informed consent of participation (Appendix 2) was also signed by all the participants. A supplementary written consent provided by the student's guardian had to be handed in if the adolescents were under the age of 16. All students and guardians were informed if any tests during the survey revealed disease or other issues that required follow-up from a doctor or a specialist.

3. Variables and data analysis

3.1 Dependent variable

Dual X-ray absorptiometry (DXA) was measured in g/cm^2 at the total hip, the femoral neck and the total body in all FF1s participants. A Z-score (units of standard deviation), which is comparing BMD and body size to what is expected in age matched adolescents, was obtained from these three measurement sites using Lunar DXA pediatric application, version 13.4. All scans were performed on the same device by specially trained personnel at UNN, following a manufactured protocol. Ten scans were excluded due to artefacts. [29]

In adults, the use of DXA is commonly is considered the golden standard to assess bone health [53]. In children and adolescents, the evidence is still unclear in regards to which skeletal site is the preferred measurement. However, there seem to be a certain consensus in the literature that the best DXA measurement for children and adolescents is the site of the lumbar spine and the total body. [54] Since FF1 had no measurement of the lumbar spine, the total body was chosen to be the main outcome variable in this analysis.

3.2 Independent variables

In the self-administered questionnaire the participants were asked questions about their dietary habits. This included consumption frequency of 14 different foods and 10 different beverages. Relevant variables to look at in regards to bone mass was the intake of calcium through milk consumption and cheese, fat fish (Vitamin D), sweets/chocolate, fruit juice, artificial sweetened beverages (diet soft drinks), and our main variables sugar sweetened and carbonated beverages.

Already known possible influential confounders to bone mass like age, BMI, sexual maturation/puberty, contraceptives, physical activity level, screen time, medication, chronic diseases, ethnicity, and the use of substances like snuff, smoking and alcohol were also included in this study. A variable about adolescent's main high school program was used as a proxy for the students own socio-economic status, to see if this had any influence on BMD.

3.2.1 Physiological and Sociodemographic variables

Age at screening: The variable was kept as a continuous variable in the analysis.

BMI: The BMI variable was calculated by dividing weight (kg) with the square of height in meters (kg/m^2), The variable was kept continuous since preliminary analyses showed a linear association.

Sexual maturity girls: The girls were asked; "If you have started menstruating, how old were you when you had your first menstruation?". The menarche age was then divided into three categories of sexual maturation; "early" (<12.5 years at menarche), "intermediate" (12.5 -13.9 years), or "late" (≥ 14 years at menarche).

Sexual maturity boys: The boys had to answer questions about four sexual maturity components; growth spurt, the growth of pubic hair, facial hair growth, and voice change on a scale from 1 (have not begun) to 4 (completed). A new variable for pubertal status was created based on the validated Pubertal Development Scale (PDS) developed by Petersen. [55] To keep the original range 1-4, the four components were summarized and then divided by 4. Answers were then categorized into "have not begun" (<2), "barely started" (2-2.9), "definitely underway" (3-3.9) or "development completed" (4). Due to the fact that the PDS-questions was implemented in a later stage of the survey a lot of the boys (n = 97) did not get the chance to answer these questions. One additional category called "not asked" was therefore created for these missing values.

Physical activity level: In regards to physical activity, the participants of the survey were asked "are you actively doing sports or physical activity outside of school?" with the alternatives "yes" and" no". If the participants answered yes to this question they were asked further; "How many hours a week are you active?" with the alternatives "none", "about half an hour", "about 1-1,5 hour", "about 2-3 hours", "about 4 to 6 hours" and "7 hours or more". These two questions combined into a new variable called "level of physical activity" with 4 new categories; "sedentary", "moderate", "active" and "very active". Those who had answered "no" to the first question were assigned to the category "sedentary". Also those who answered yes to the first question, but "none" to the question regarding hours of activity were assigned to this category. Participants who had answered "half an hour" and "about 1-1,5 hours" were assigned to the category "moderate". Participants who had answered "about 2-3 hours" and "about 4-6 hours" were merged together and put in the category "active". And lastly, those who had answered "7 hours or more" were put in the category "very active".

Daily Screen time: Participants were asked about how many hours per day they spend watching PC, TV, DVD etc. outside of school during weekdays, and a similar question for weekends. They could choose between the categories ”none”, ”about 30 min”, ”about 1 hour – 1 hour and 30 min”, ”about 2-3 hours”, ”about 4-6 hours”, ”about 7-9 hours” or ”10 hours or more”. A single variable for the daily average screen time outside of school was created based on the mean values for each of the intervals. The mean value for daily screen time for school days was weighted differently (5/7) than non-school days (2/7). The new variable was divided into the categories ”up to 2 hours”, ”between 2-4 hours”, and ”4 hours and more”.

Alcohol use: Participants were asked ”How often do you drink alcohol?” with the categories ”never”, ”once per month or less”, ”2-4 times per month”, ”2-3 times per week” and ”4 or more times per week”. Here the last three categories were merged together into one category due to small groups and named ”2 or more times per month”.

Smoking and snuff use: The participants were asked about tobacco use, and the questions was; ”Do you smoke?” and “Do you snuff?” with three alternatives; “daily”, “sometimes” or “never”. The category” daily” was merged together with” sometimes” due to few respondents in these two groups for both the smoking and snuff variable.

Medication known to affect bone: Medication that is known to cause bone loss was selected based on the Anatomical Therapeutic Chemical Index (ATC index), which is an international classification system for active ingredients of drugs. A new variable was created called ”medication known to affect bone” with categories ”yes” and ”no”. Participants who used medication with ATC index; D07A (Corticosteroids), H03A (Thyroid preparations), N03A (Antieleptics), R01AD (Corticosteroids) and R03BA (Glucocorticoids) were assigned to the ”yes” category. [29]

Chronic diseases known to affect bone: In the interview the participants were asked if they had any chronic diseases. A new variable called ”disease known to affect bone” was created with the categories ”yes” and ”no”. The international Classification of disease (ICD-10) codes were used to extract the relevant diseases. The participants who had chronic diseases with ICD codes; E03 (Hypothyreosis), E10 (Type 1, Diabetes mellitus), F50.9 (Eating disorders), M13.4 (Arthritis), and K90 (Celiac disease) were assigned to the ”yes” category. [29]

Main high school program: In the survey the main high school programs were classified as ”program for specialization in general studies”, ”program for sports and physical education” and ”vocational program”.

3.2.2 Beverage variables

In consumption of the different drink beverages, the participants were asked ”how much do you drink of the following?” and then listed the different beverage-alternatives. The response alternatives were ”seldom/never”, ”1-6 glass per week”, ”1 glass per day”, ”2-3 glass per day”, and ”4 glasses or more per day” on each item. To find the daily consumption for all drink variables, all the answers were recoded into the average number of glasses the participant would approximately consume on a daily basis; seldom/never = 0, ”1-6 glass per week” = 0.5, ”1 glass per day” = 1, ”2-3 glass per day” = 2.5, and ”4 glasses or more per day = 4”.

Artificial sweetened beverages and Sugar sweetened beverages: The participants were also asked about the consumption of squash with sugar, artificial sweetened squash, soft drinks with sugar, and artificial sweetened soft drinks (The term “artificial sweetened” includes drinks with reduced sugar content or sugar replacement). The variables” squash with sugar” and” soft drinks with sugar” were added together into one new variable called” sugar

sweetened beverages", and then divided into the categories "None", "half a glass daily", "1-1,5 glass daily" and "2 or more glasses daily". "Artificial sweetened squash" and "artificial sweetened soft drink" were added together and created into the new variable "artificial sweetened beverages". This variable was divided into the response categories "none", "half a glass daily", "1 glass daily" and "more than 1 glass daily".

Carbonated beverages: Since former research has suggested that carbonated soft drinks can have a negative impact on bone mineral density, a new variable named "carbonated beverages" were also created by adding the variables "artificial sweetened soft drink" and "soft drink with sugar" together. This variable was divided into the categories "none", "half a glass daily", "1-1.5 glass daily" and "2 or more glasses daily".

3.2.3 Nutritional variables

Daily milk Consumption: The participants were asked about four different types of milk; whole fat milk, reduced fat milk, skimmed milk and extra reduced fat milk. Since the level of calcium in milk is the same regardless of what type of milk you drink, the four items were added together into one variable called "daily milk consumption". 4 new categories were created; "none or up to half a glass daily", "1-1.5 glass daily", "2-3 glasses daily", and "more than 3 glasses daily". The categories were created like this to have a more equal frequency of participants in each group.

Cheese: Participants were asked "How frequently do you usually eat cheese (all kinds)?" with the alternatives "rarely/never", "1-3 times per month", "1-3 times per week", "4-6 times per week", and "every day". Here the categories "4-6 times per week" and "every day" were added together into one category renamed "4-7 times a week" due to few respondents.

Fat Fish: Participants were asked ”How often do you usually eat fat fish (e.g. salmon, trout, mackerel, herring)?” with the categories ”rarely/never”, ”1-3 times per month”, ”1-3 times per week”, ”4-6 times per week”, ”every day”. Due to small groups, the categories ”4-6 times per week” and” every day” were merged together into a new category called ”4-7 times per week”.

Fruit Juice: Participants were asked “How often do you drink fruit juice”? with following categories “rarely/never”, “1-6” glasses a week”, “1 glass daily”, “2-3 glasses daily” and “4 glasses or more daily”. The variable was recoded into the average number of glasses the participant would approximately consume on a daily basis, and 4 new categories was created for this new variable; “none”, “Half a glass daily“, “1 glass daily”, and “2.5 glasses or more daily”.

Chocolate/sweets: The participants were asked” How often do you usually eat sweets? (e.g. chocolate, candy)”, with the categories ”rarely/never”, ”1-3 times per month”, ”1-3 times per week”, ”4-6 times per week”, and ”every day”. Due to small groups, the two categories ”4-6 times per week” and” every day” were merged together into a category called ”4-7 times per week”.

3.3 Statistics

To best answer our research-question we chose to conduct a multiple regression analysis. All analyses were performed using the Statistical Package of Social Sciences software (SPSS V.24), and all two-sided p-values of <0.05 were considered statistically significant. Gender stratification was conducted on all the main analysis due to the known variation in bone mass acquisition between boys and girls [56].

A descriptive analysis was first performed to find the baseline characteristics. The association between confounding variables and BMD total body was further explored in a univariate regression model, and here we included variables with a P value ≤ 0.25 for further analysis.

A manual hierarchical block analysis was then performed to select the variables to best explain the dependent variable from a large set of predictors. The variables were manually entered in blocks into the model, and we choose this method of selection due to a large number of categorical variables with coded dummy variables in our dataset. The variables were entered into blocks based on current knowledge of importance and theoretical background. The hierarchical block analysis was also adjusted for sex, and the block of variables that had a significant contribution (sig. F change) to the model were included further in the main analysis. In the main analysis, a multiple regression analysis for the BMD variable total body in relation to sugar sweetened beverages and carbonated beverages was conducted with adjustment for confounding variables found in the block analysis.

We also checked for normal distribution, violations of assumptions, and the correlation between bone mass and the different determinants. The assumptions of linearity, normal distribution, variance heterogeneity and multicollinearity were assessed using histogram,

normal P-P plot, residual plots and variance inflation tests. No violations of the assumptions for multiple regression analysis were found.

4. Findings

4.1. Baseline characteristics

The population in this master thesis consisted of 440 girls and 460 boys aged from 15-17 years of age. The average age of the participants was 16 years old for both girls and boys. The mean values for bone mass total body (girls 1.14 g/cm and boys 1.17 g/cm) and BMI (Girls 22.36, boys 22.40) for both sexes (see table 1) were very similar at baseline.

In regards to sexual maturation, 31.4 % of the girls reported to have started their sexual maturation early, which means they were younger than 12.5 years when got their menarche. In comparison, 59.3 % the boys reported to have completed their pubertal development (the category "not asked" is included when presenting the % of participants here). The majority of girls (46.4%) reported to be intermediate in relation to sexual maturation (Boys 13 %).

Most of the girls had chosen general studies as their main high school program, while the majority of boys studied vocational program

Around 1/3 of all the adolescents reported a physical activity level of sedentary. There was a higher prevalence of girls that reported to be active than boys (girls 45.7% and boys 35.7%), but more boys that reported to be very active compared to girls (boys 20% and girls 13.6%). In relation to screen time, boys spent more time watching PC, DVD and TV. 47.2 % of the boys said they spent 4 hours or more in front of a screen.

In general, most of the adolescents reported to not drink alcohol at all, or in moderate amounts. Approximately 1/3 of the adolescents reported to drink twice or more per month (Girls 29.8% and Boys 30.2%). In total, more of the girls (76.8%) reported to drink alcohol compared to boys (67.8%).

Approximately 1/5 of the adolescents reported to smoke sometimes/daily. There were more adolescents that used snuff compared to cigarettes, with 33.2 % of the girls and 40.9 % of the boys reporting to snuff sometimes/daily.

63.6 % of the girls reported to use contraceptives.

Table 1: *Physiological and lifestyle characteristics of participants in the Fit Futures Study in relation to gender.*

Characteristics	Girls (N = 440)	Boys (N = 460)
BMD total body, mean g/cm^2 (sd)	1.14 (0.07)	1.17 (0.09)
Age at screening, mean age (sd)	16.12 (0.40)	16.07 (0.44)
BMI, mean (sd)	22.36 (3.98)	22.40 (4.20)
Sexual maturity girls, % (n)		
Early	31.4 (138)	
Intermediate	46.4 (204)	
Late	22.3 (98)	
Sexual maturity boys, % *(%) (n)		
Development completed		59.3 *(75.3) (273)
Definitely underway		13.0 *(16.4) (60)
Barely started		6.5 *(8.3) (30)
Not asked		21.1 (97)
Main High School program, % (n)		
General studies	53.2 (234)	30.7 (141)
Sports and physical education	8.6 (38)	13.5 (62)
Vocational program	38.2 (168)	55.9 (257)
Physical activity level, % (n)		
Sedentary	30.9 (136)	34.3 (158)
Moderate	9.8 (43)	10.0 (46)
Active	45.7 (201)	35.7 (164)
Very active	13.6 (60)	20.0 (92)
Screen time outside of school, % (n)		
Up to two hours	25.5 (112)	14.3 (66)
Between 2 and 4 hours	39.5 (174)	38.5 (177)
4 hours or more	35.0 (154)	47.2 (217)
Alcohol use, % (n)		
Never	23.2 (102)	32.2 (148)
Once per month	47.0 (207)	37.6 (173)
Twice or more per month	29.8 (131)	30.2 (139)
Smoke use, % (n)		
No, never	80.2 (353)	77.0 (354)
Sometimes/daily	19.8 (87)	23.0 (106)
Snuff use, % (n)		
No, never	66.8 (294)	59.1 (272)
Sometimes/daily	33.2 (146)	40.9 (188)
Contraceptive use girls, % (n)		
Yes	63.6 (280)	
No	36.4(160)	

** % of participants in the different sexual maturity categories for boys without including participants in the category "Not asked".*

Concerning drinking sugar sweetened beverages (see table 2) there was a clear difference in habits between the genders. 29.3% of the girls reported to never drink, whereas only 8.7% of the boys reported the same. 23.3% of the boys reported drinking 2 or more glasses daily, where only 8.2 % of the girls said the same.

In relation to drinking carbonated beverages, the boys also had a higher consumption. 22.3 % of the girls said they never drank carbonated beverages, but only 6.1 % of the boys answered the same. A small minority of girls (8.9 %) said they drank 2 or more glasses daily, while 23.7 % of the boys reported that they did.

In regards to consumption of artificial sweetened beverages around 40 % of the boys and girls reported to never drink artificial sweetened beverages, around 30 % reported half glass daily, and approximately 20 % more than 1 glass.

In relation to the other dietary variables (see table 2), boys scored the highest on consumption of milk and cheese. 27 % of the boys reported to drink more than 3 glasses of milk daily. Most of the girls reported to drink 1-1.5 glass daily. 42.8 % of the boys ate cheese 4-7 times a week, and 42.7 % of the girls reported to eat cheese 1-3 times a week.

Approximately 50 % of all the adolescents reported to drink half a glass of fruit juice every day, eat fat fish 1-3 times per month, and eating sweets/chocolate 1-3 times per week.

The variables “medication known to affect bone”, “diseases known to affect bone”, and “ethnicity” were excluded from further analysis due to very small groups in relation to the categories non-white (1.6 %), participants with a disease (0.9 %), or users of medication (2.5 %).

Table 2: *Dietary intake of participants in the fit future study in relation to gender.*

Variables	Girls (N = 440)	Boys (N = 460)
Sugar sweetened beverages, % (n)		
None	29.3 (129)	8.7 (40)
Half a glass daily	38.4 (169)	31.1 (143)
1-1,5 glass daily	24.1 (106)	37.0 (170)
2 or more glasses daily	8.2 (36)	23.3 (107
Carbonated beverages, % (n)		
None	22.3 (98)	6.1 (28)
Half a glass daily	35.2 (155)	36.3 (167)
1-1,5 glass daily	33.6 (148)	33.9 (156)
2 or more glasses daily	8.9 (39)	23.7 (109)
Artificial sweetened beverages, % (n)		
None	39.3 (173)	42.4 (195)
Half a glass daily	31.8 (140)	27.8 (128)
1 glass daily	20.0 (88)	17.6 (81)
More than 1 glass daily	8.9 (39)	12.2 (81)
Milk % (n)		
None or up to half a glass daily	25.7 (113)	23.9 (110)
1-1,5 glass daily	31.6 (139)	24.3 (112)
2-3 glass daily	25.5 (112)	24.8 (114)
More than 3 glasses daily	17.3 (76)	27.0 (124)
Cheese, % (n)		
Rarely/never	4.8 (21)	4.3 (20)
1-3 times per month	17.3 (76)	14.6 (67)
1-3 times per week	42.7 (188)	38.3 (176)
4-7 times per week	35.2 (155)	42.8 (197)
Fruit Juice, % (n)		
None	15.7 (69)	16.5 (76)
Half a glass daily	49.5 (218)	46.3 (213)
1 glass daily	19.5 (86)	22.2 (102)
2.5 glasses or more daily	15.2 (67)	15.0 (69)
Fat fish, % (n)		
Rarely/never	19.8 (87)	22.6 (104)
1-3 times per month	50.0 (220)	45.7 (210)
1-3 times per week	26.1 (115)	27.2 (125)
4-7 times per week	4.1 (18)	4.6 (21)
Sweets/chocolate, % (n)		
Rarely/never	5.9 (26)	7.0 (32)
1-3 times per month	23.4 (103)	25.0 (115)
1-3 times per week	56.6 (249)	55.4 (255)
4-7 times per week	14.1 (62)	12.6 (58)

4.2 Univariate associations between variables previously associated with BMD

The association between confounding variables and BMD total body were first explored in univariate regression analysis. In relation to physiological/sociodemographic variables (see table 3), the higher values of the continuous variables age and BMI had strong significant associations with higher bone mass density ($p<0.01$).

Sexual maturity was also related to BMD. Adolescents who reported to start their sexual maturity early, or were fully developed, had higher bone mass (girls 0.019 g/cm^2, boys 0.033 g/cm^2) compared to the adolescents who were intermediate or well underway. Late development was associated with lower values of BMD (girls -0.030 g/cm^2, boys -0.043 g/cm^2) compared to the reference category.

Students who had chosen sports and physical education as their main high school program, had higher values of BMD (girls 0.033 g/cm^2, boys 0.054 g/cm^2) compared to students who had chosen general program. For the girls choosing vocational programs, lower values of BMD were found (-0.017 g/cm^2).

The two highest levels of physical activity, active and very active, had a positive association with BMD for both sexes compared to the students who reported sedentary. In relation to screen time, spending 4 hours or more in front a screen based modality showed lower values (-0.031 g/cm^2) of BMD for boys.

Variables like smoking, snuffing, and alcohol had no significant relationship ($p<0.05$) to BMD total body

Table 3: *Associations between physiological/sociodemographic variables and BMD total body[1]. Analysis is stratified by sex.*

	Girls (N = 440)		**Boys (N = 460)**	
Variables	**Unstandardized B (CI)**	**P-value**	**Unstandardized B (CI)**	**P-value**
Age (years)	0.021 (0.004 – 0.039)	**0.017**	0.025 (0.005 – 0.045)	**0.013**
BMI (kg/m2)	0.009 (0.007 – 0.011)	**0.000**	0.011 (0.009 – 0.013)	**0.000**
Sexual maturity girls				
Early	0.019 (0.003 – 0.035)	**0.021**		
Intermediate	Reference			
Late	-0.030 (-0.048 - -0.013)	**0.001**		
Puberty Boys				
Development completed			0.033 (-0.002 – 0.068)	**0.067**
Well underway			Reference	
Barely started			-0.043 (-0.069 - -0.017)	**0.001**
Not asked			-0.030 (-0.052 - -0.008)	**0.007**
Main High school program				
Specialization in general studies	Reference		Reference	
Sports and physical education	0.033 (0.008 – 0.059)	**0.010**	0.054 (0.026 – 0.082)	**0.000**
Vocational program	-0.017 (-0.031 – -0.002)	**0.027**	-0.012 (-0.032 – 0.007)	0.202
Physical activity level				
Sedentary	Reference		Reference	
Moderate	0.007 (-0.019 – 0.033)	0.588	-0.016 (-0.047 – 0.014)	0.287
Active	0.023 (0.007 – 0.039)	**0.006**	0.037 (0.017 – 0.058)	**0.000**
Very active	0.042 (0.019 – 0.064)	**0.000**	0.058 (0.034 – 0.082)	**0.000**
Screen time outside school				
Up to two hours	Reference		Reference	
Between 2 and 4 hours	0.009 (-0.008 – 0.027)	0.302	-0.004 (-0.031 – 0.023)	0.762
4 hours or more	-0.005 (-0.023 – 0.014)	0.630	-0.031 (-0.057 - -0.005)	**0.020**
Alcohol use				
Never	Reference		Reference	
Once per month or less	0.004 (-0.014 – 0.022)	0.677	0.020 (-0.001 – 0.041)	0.062
Twice or more per month	-0.001 (-0.021 – 0.018)	0.855	0.012 (-0.010 – 0.034)	0.292
Smoking				
No, never	Reference		Reference	
Sometimes/daily	-0.010 (-0.028 – 0.007)	0.250	-0.010 (-0.031 – 0.011)	0.339
Snuff use				
No, never	Reference			
Sometimes/daily	0.000 (-0.015 – 0.015)	0.988	0.015 (-0.002 – 0.033)	0.90

[1]*Univariate regression analysis. Significance level is set to p≤ 0.05 (marked in* ***bold****). Variables with p ≤ 0.25 were tested further in the block-analysis.*

In regards to the various dietary variables (see table 4), not drinking any Sugar sweetened beverages (SSB) had a significant positive association with BMD total body for girls (0.027 g/cm^2) compared to girls who reported drinking half a glass daily.

For the adolescents reporting to never drink artificial sweetened beverages, there was found a significant association to bone mass with lower BMD values compared to drinking half a glass daily. Regarding carbonated beverages (CB), no significant association with BMD total body was found.

Rarely eating sweets/chocolate was associated with higher values of BMD for girls (0.118 g/cm^2) compared to the girls who ate sweets/chocolate 1-3 times a week. Boys reporting to never/rarely eat cheese had lower values of BMD (-0.065 g/cm^2) compared to the reference category 1-3 times a week.

No statistical significant results were found for the other dietary variables.

The variables contraceptive and fruit juice were excluded after univariate regression analysis due to no associations ($p \leq 0.25$) with bone mineral density.

Table 4: *Associations between dietary variables and BMD total body[1]. Analysis is stratified by sex.*

	Girls (N = 440)		**Boys (N = 460)**	
Variables	**Unstandardized B (CI)**	**P-value**	**Unstandardized B (CI)**	**P-value**
Sugar sweetened beverages				
None	0.027 (0.010 – 0.044)	**0.002**	0.009 (-0.025 – 0.042)	0.611
Half a glass daily	Reference		Reference	
1-1.5 glass daily	-0.002 (-0.020 – 0.017)	0.862	-0.010 (-0.031 – 0.011)	0.354
2 or more glasses daily	-0.010 (-0.037 – 0.017)	0.468	-0.020 (-0.044 – 0.003)	0.093
Carbonated beverages				
None	0.004 (-0.015 – 0.023)	0.699	0.008 (-0.030 – 0.047)	0.663
Half a glass daily	Reference		Reference	
1-1.5 glass daily	-0.015 (-0.032 – 0.002)	0.080	0.016 (-0.005 – 0.036)	0.144
2 or more glasses daily	0.003 (-0.024 – 0.029)	0.849	-0.008 (-0.031 – 0.015)	0.519
Artificial sweetened beverages				
None	-0.025 (-0.042 - -0.008)	**0.004**	-0.026 (-0.047 – -0.004)	**0.018**
Half a glass daily	Reference		Reference	
1-1.5 glass daily	-0.017 (-0.37 – 0.003)	0.094	-0.018 (-0.045 – 0.008)	0.178
2 or more glasses daily	-0.009 (-0.036 – 0.018)	0.509	-0.012 (-0.042 – 0.018)	0.439
Glass of milk daily				
None or up to half a glass daily	Reference		Reference	
1-1,5 glass daily	-0.010 (-0.029 – 0.009)	0.294	0.015 (-0.010 – 0.041)	0.229
2 – 3 glasses daily	0.016 (-0.003 – 0.036)	0.107	0.002 (-0.023 – 0.027)	0.856
More than 3 glasses daily	0.011 (-0.011 – 0.032)	0.341	0.022 (-0.002 – 0.047)	0.078
Cheese				
Rarely/never	0.016 (-0.018 – 0.050)	0.368	-0.065 (-0.109 - -0.021)	**0.004**
1-3 times per month	-0.012 (-0.032 – 0.008)	0.240	-0.002 (-0.029 – 0.024)	0.867
1-3 times per week	Reference		Reference	
4-7 times per week	0.008 (-0.008 – 0.024)	0.322	-0.006 (-0.025 – 0.014)	0.566
Fruit Juice				
None	0.004 (-0.017 – 0.024)	0.709	-0.014 (-0.039 – 0.011)	0.264
Half a glass daily	Reference		Reference	
1 glass daily	-0.006 (-0.025 – 0.013)	0.554	0.005 (-0.017 – 0.028)	0.654
2.5 glass or more daily	0.004 (-0.017 – 0.024)	0.736	-0.007 (-0.033 – 0.019)	0.591
Fat fish				
Rarely/never	-0.012 (-0.033 – 0.009)	0.277	-0.010 (-0.035 – 0.014)	0.411
1-3 times per month	-0.015 (-0.032 – 0.002)	0.091	0.011 (-0.010 – 0.033)	0.288
1-3 times per week	Reference		Reference	
4-7 times per week	-0.001 (-0.039 – 0.036)	0.953	0.000 (-0.045 – 0.044)	0.984
Sweets/chocolate				
Rarely/never	0.037 (0.006 – 0.067)	**0.018**	0.006 (-0.029 – 0.041)	0.724
1-3 times per month	0.015 (-0.003 – 0.032)	0.097	0.012 (-0.009 – 0.033)	0.271
1-3 times per week	Reference		Reference	
4-7 times per week	-0.003 (-0.024 – 0.018)	0.774	-0.015 (-0.043 – 0.012)	0.272

[1]*Univariate regression analysis. Significance level is set to p≤ 0.05 (marked in* ***bold****). Variables with p ≤ 0.25 were tested further in the block-analysis.*

4.3 Hierarchical Block Analysis

In the block analysis (see table 5) the variables that were considered of biological importance to control for were entered first in the model (sex, age, BMI and sexual maturation). Sociodemographic (main high school program) and behavioral variables (physical activity, screen time, alcohol, snuff, and smoking) considered to have a positive or negative influence on BMD were entered next in blocks. After that, blocks related to nutrition were entered into the model. Here Milk and cheese were selected together in one block due the positive effect that calcium has on BMD. Fat Fish was entered in one block due to the positive effects of Vitamin D, and in the last block the rest of the nutritional variables were entered together (artificial sweetened beverages and sweets/chocolate).

Each new block was entered one by one. The block entered was removed before the next block was entered if the variables did not contribute to a significant F change to the model. If the block contributed to a significant F change it was kept in the model, and then a new block was then entered.

Age, BMI, sexual maturity, main high school program, physical activity, daily screen time, alcohol use, and artificial sweetened beverages gave a significant change or contribution to the model. These variables were kept further in the main analysis. Artificial sweetened beverages were only adjusted for in the multivariate analysis where we investigated the associations between sugar sweetened beverages and BMD total body.

Table 5: *Manual Hierarchical Stepwise Block analysis between confounding variables and BMD total body.*

Block	R Square	R Square Change	Sig. F Change
1. Sex, age, BMI, sexual maturity boys & girls	0.297	0.297	0.000
2. Main high school program	0.354	0.057	0.000
3. Physical activity level, daily screen time	0.385	0.031	0.000
4. Alcohol use, smoking, snuff	0.391	0.006	0.061**
5. Alcohol use	0.390	0.005	0.021
6. Milk, cheese	0.397	0.007	0.140 *
7. Fat fish	0.392	0.001	0.552 *
8. Artificial sweetened beverages, sweets/chocolate	0.397	0.006	0.159 ***
9. Artificial sweetened beverages	0.395	0.005	0.067

** Block excluded from the model.*

*** The block was not a significant contribution to the model, but the variable Alcohol had a significant p-value in one the categories in the coefficient table. The variable was therefore tested further in a block alone (block 5). Since the F-change of the new block had a significant contribution to the model, the variable was kept further in the analysis.*

**** The block was not a significant contribution to the model, but the variable artificial sweetened beverages had a significant p-value in one of categories in the coefficient table. The variable was therefore tested further in a block alone (block 9). Since the F-Change of the new block was borderline significant to the model, we chose to keep the variable further in the analysis.*

4.4 Multivariate associations between soft drink beverages and BMD total body.

Presentation of result from the main analysis. All analysis is stratified by sex and adjusted for the confounding variables found in the block analysis.

4.4.1 Associations between sugar sweetened beverages and BMD total body.

For girls who reported to not drink SSB there was found a significant association with higher BMD values (0.016 g/cm^2) compared to girls drinking half a glass daily. No other significant associations were found between SSB and BMD total body (see table 6 and 7). Girls who reported to never drinking artificial sweetened beverages had a significant association to BMD total body, with lower BMD values (-0.016 g/cm^2) compared to the reference group "half a glass daily".

In regards to the biological variables, one unit higher age or BMI was associated with higher bone mass for the adolescents, however age was only significant for girls when adjusting for the other variables. In relation to sexual maturity, girls (see table 6) who started developing early had higher values of BMD (0.010 g/cm^2) compared to girls reporting intermediate. Girls who developed late had lower values (-0.021 g/cm^2). Boys who reported to barely have started puberty (0.060 g/cm^2) or completed their development (0.041 g/cm^2), both had higher values of BMD compared to the reference group "definitely underway".

In the other variables, results showed the same trend as in the univariate analysis. Girls and boys who had chosen sports and physical education as main high school program had higher BMD values compared to the reference group (girls 0.026 g/cm^2, boys 0.038 g/cm^2). The boys who had chosen vocational program also had a significantly lower BMD- value (-0.021 g/cm2) compared to those in general studies. Physical active adolescents, meaning those who reported 2-6 hours'(active), or more than 7 hours (very active) of activity outside of school,

had significantly higher BMD values in both genders. Daily screen time and alcohol only had a significant association for boys. 4 hours or more using sedentary screen based modalities gave lower BMD values (-0.025 g/cm^2), and more surprisingly, drinking twice a month or more often was associated higher BMD levels for boys (0.024 g/cm^2).

If we look only at the Beta values (standardized coefficients) from the result, BMI is the most influential predictor in the analysis for both genders (Girls Beta 0.479, Boys Beta 0.518), For girls, the physical activity level "very active" is the second most influential predictor (Beta 0.140), followed by late sexual maturity (Beta -0.117). For boys, the puberty levels "development completed" (Beta 0.212) and "barely started" (Beta 0.155) were most influential after BMI.

In regards to the summary of the model, 36.4 % of the total variance in BMD total body (R square) was explained by the predictor variables for girls, and 41.2 % of the total variance was explained by predictor variables for boys. When adjusted for the number of predictors in the model (adjusted R square), the total variance explained by predictor variables was changed to 33.5 % for girls, and 38.6 % for boys.

Table 6: *Associations[1] between sugar sweetened beverages and bone mineral density among girls (N 440) in the Fit Futures study, with adjustment for confounding variables in a multivariate model.*

Variables	Unstandardized B	Beta	Sig. (P<0.05)	CI unstand.B
Age	0.021	0.113	**0.005**	0.007 – 0.036
BMI	0.009	0.479	**0.000**	0.008 – 0.011
Sexual maturity				
Early	0.010	0.064	0.135	-0.003 – 0.024
Intermediate	Reference	Reference	Reference	Reference
Late	-0.021	-0.117	**0.006**	-0.036 - - 0.006
Main high school program				
General studies	Reference	Reference	Reference	Reference
Sports and physical education	0.026	0.097	**0.025**	0.003 – 0.049
Vocational program	-0.013	-0.082	0.069	-0.027 – 0.001
Physical activity				
Sedentary	Reference	Reference	Reference	Reference
Moderate	-0.002	-0.008	0.862	-0.024 – 0.020
Active	0.016	0.104	**0.035**	0.001 – 0.030
Very active	0.031	0.140	**0.005**	0.009 – 0.052
Daily Screen time				
Up to 2 hours	Reference	Reference	Reference	Reference
Between 2 – 4 hours	0.012	0.076	0.128	-0.003 – 0.027
4 hours or more	0.003	0.020	0.701	-0.013 – 0.019
Alcohol use				
Never	Reference	Reference	Reference	Reference
Once per month or less	0.001	0.008	0.869	-0.014 – 0.016
Twice or more per month	0.001	0.007	0.896	-0.015 – 0.017
Artificial sweetened beverages				
None	-0.016	-0.102	**0.031**	-0.030 - -0.001
Half a glass daily	Reference	Reference	Reference	Reference
1 glass daily	-0.016	-0.085	0.071	-0.033 – 0.001
More than 1 glass daily	-0.001	-0.004	0.928	-0.025 – 0.023
Sugar sweetened beverages				
None	0.016	0.099	**0.029**	0.002 – 0.031
Half a glass daily	Reference	Reference	Reference	Reference
1-1,5 glass daily	0.002	0.013	0.783	-0.014 – 0.018
2 or more glasses daily	0.002	0.006	0.890	-0.023 – 0.027

[1] *Multiple regression analysis. Significance level p<0.05.*

Model summary: R square = 0.364, Adjusted R square = 0.335.

Table 7: *Associations[1] between sugar sweetened beverages and bone mineral density among boys (N 460) in the Fit Futures study, adjusted for confounding variables in a multivariate model.*

Variables	Unstandardized B	Beta	Sig. (P<0.005)	CI unstand.B
Age	0.015	0.070	0.067	-0.001 – 0.031
BMI	0.012	0.518	**0.000**	0.010 – 0.013
Sexual maturity boys				
Development completed	0.041	0.212	**0.000**	0.020 – 0.063
Definitely underway	Reference	Reference	Reference	Reference
Barely started	0.060	0.155	**0.001**	0.026 – 0.093
Not asked	0.022	0.093	0.095	-0.004 – 0.047
Main high school program				
General studies	Reference	Reference	Reference	Reference
Sports and physical Education	0.038	0.135	**0.004**	0.012 – 0.063
Vocational program	-0.021	-0.112	**0.016**	-0.039 - -0.004
Physical activity				
Sedentary	Reference	Reference	Reference	Reference
Moderate	-0.009	-0.027	0.500	-0.034 – 0.017
Active	0.028	0.143	**0.001**	0.011 – 0.045
Very active	0.035	0.147	**0.004**	0.011 – 0.059
Daily Screen time				
Up to 2 hours	References	References	References	References
Between 2 – 4 hours	-0.006	-0.031	0.581	-0.028 – 0.016
4 hours or more	-0.025	-0.129	**0.026**	-0.046 - -0.003
Alcohol use				
Never	Reference	Reference	Reference	Reference
Once per month or less	0.016	0.081	0.070	-0.001 – 0.033
More than twice a month	0.024	0.115	**0.011**	0.005 – 0.042
Artificial sweetened beverages				
None	-0.008	-0.044	0.329	-0.026 – 0.009
Half a glass daily	References	References	References	References
1 glass daily	-0.004	-0.015	0.722	-0.025 – 0.017
More than 1 glass daily	0.003	0.011	0.787	-0.021 – 0.028
Sugar sweetened beverages				
None	0.000	0.000	0.993	-0.027 – 0.027
Half a glass daily	Reference	Reference	Reference	Reference
1-1,5 glass daily	-0.007	-0.035	0.444	-0.024 – 0.011
2 or more glasses daily	0.004	-0.019	0.676	-0.024 – 0.016

[1] *Multiple regression analysis. Significance level p<0.05.*

Model summary: R square = 0.412, Adjusted R Square = 0.386..

4.4.2. Associations between carbonated beverages and BMD total body.

In regards to CB we found no significant association with BMD total body for either girls or boys (see table 8 and 9).

For the other confounding variables, we observed the same pattern in this analysis as with SSB in the other main analysis. For girls (see table 8), BMI (0.009 g/cm^2) and age (0.020 g/cm^2) were positive predictors, showing higher BMD values per unit increase in these two predictors. BMI was also a positive predictor for boys (0.012 g/cm^2). Late sexual maturity for girls was significantly associated with lower values of BMD (-0.023 g/cm^2). For boys (see table 9), a significant association with "completed development" (0.042 g/cm^2) and "barely started" puberty (0.061 g/cm^2) was associated with higher values of BMD.

In regards to main high school program, sports and physical education (girls 0.025 g/cm^2, boys 0.031 g/cm^2) had a positive significant association, while vocational program (girls -0.015, boys -0.020 g/cm^2) was associated with lower values of BMD for both genders.

In relation to physical activity, levels active and very active gave higher values of BMD for both genders in comparison to the sedentary adolescents. Only the boys who watched TV or played computer for 4 hours or more every day had significant lower values of BMD (-0.027 g/cm^2), compared to the boys who watched up to 2 hours daily. Boys who drank alcohol twice or more per month also had higher values of bone mass (0.023 g/cm^2) in comparison to the reference category.

35.1 % of the total variance (R-square) in BMD whole body for girls, and 41.1 % for boys, was explained by the predictor variables. When adjusted for the number of predictors in the model (adjusted R square), the total variance was modified to 32.7 % for girls, and 38.8 % for the boys. Looking at the Beta values (see table 8 and 9), we can see that BMI is the most influential predictor for BMD total body for both girls and boys.

Table 8: *Associations[1] between carbonated beverages and bone mineral density among girls (N 440) in the Fit Futures study, adjusted for confounding variables in a multivariate model.*

Variables	Unstandardized B	Beta	Sig. (P<0.05)	CI unstand.B
Age	0.020	0.109	**0.007**	0.006 – 0.035
BMI	0.009	0.489	**0.000**	0.008 – 0.011
Sexual maturity				
Early	0.010	0.061	0.154	-0.004 – 0.024
Intermediate	Reference	Reference	Reference	Reference
Late	-0.023	-0.125	**0.004**	-0.038 - -0.007
Main high school program				
General studies	Reference	Reference	Reference	Reference
Sports and Education	0.025	0.094	**0.029**	0.003 – 0.048
Vocational program	-0.015	-0.099	**0.029**	-0.029 – -0.002
Physical activity				
Sedentary	Reference	Reference	Reference	Reference
Moderate	0.000	0.001	0.979	-0.022 – 0.022
Active	0.016	0.107	**0.031**	0.002 – 0.031
Very active	0.033	0.152	**0.003**	0.012 – 0.055
Daily Screen time				
Up to 2 hours	Reference	Reference	Reference	Reference
Between 2 – 4 hours	0.014	0.090	0.073	-0.001 – 0.029
4 hours or more	0.004	0.025	0.625	-0.012 – 0.020
Alcohol use				
never	Reference	Reference	Reference	Reference
Once per month or less	0.005	0.031	0.544	-0.10 – 0.020
More than twice a month	0.005	0.027	0.588	-0.012 – 0.021
Carbonated beverages				
None	-0.001	-0.008	0.868	-0.018 - -0.015
Half a glass daily	Reference	Reference	Reference	Reference
1-1,5 glass daily	-0.012	-0.073	0.106	-0.026 – 0.002
2 or more glasses daily	-0.001	-0.005	0.913	-0.024 – 0.021

[1]*Multivariate regression analysis. Significance level p<0.05*

Model Summary: R-square = 0.351, Adjusted R Square = 0.327.

Table 9: *Associations[1] between carbonated beverages and bone mineral density among boys (N 460) in the Fit Futures study, adjusted for confounding variables in a multivariate model.*

Variables	Unstandardized B	Beta	Sig. (P<0.005)	CI unstandard. B
Age	0.014	0.066	0.081	-0.002 – 0.030
BMI	0.012	0.522	**0.000**	0.010 – 0.014
Sexual maturity				
Development completed	0.042	0.215	**0.000**	0.021 – 0.063
Definitely underway	Reference	Reference	Reference	Reference
Barely started	0.061	0.517	**0.000**	0.027 – 0.094
Not asked	0.023	0.097	0.079	-0.003 – 0.048
Main high school program				
General studies	Reference	Reference	Reference	Reference
Sports and physical education	0.031	0.122	**0.022**	0.004 – 0.057
Vocational program	-0.020	-0.110	**0.023**	-0.038 - -0.003
Physical activity				
Sedentary	Reference	Reference	Reference	Reference
Moderate	-0.011	-0.033	-0.402	-0.036 – 0.014
Active	0.029	0.146	**0.001**	0.012 – 0.046
Very active	0.034	0.175	**0.004**	0.011 – 0.058
Daily Screen time				
Up to 2 hours	Reference	Reference	Reference	Reference
Between 2 – 4 hours	-0.007	-0.037	0.510	-0.029 – 0.015
4 hours or more	-0.027	-0.139	**0.017**	-0.048 - -0.005
Alcohol use				
Never	Reference	Reference	Reference	Reference
Once per month or less	0.015	0.076	0.084	-0.002 – 0.032
More than twice a month	0.023	0.110	**0.015**	0.005 – 0.041
Carbonated beverages				
None	0.005	0.012	0.762	-0.026 – 0.035
Half a glass daily	Reference	Reference	Reference	Reference
1-1,5 glass daily	0.007	0.035	0.413	-0.010 – 0.024
2 or more glasses daily	0.008	0.035	0.413	-0.011 – 0.027

[1]*Multiple regression analysis. Significance level p<0.05.*

Model summary: R square = 0.411, Adjusted R Square 0.388.

5. Discussion of methodological considerations

In every study design errors can occur either by chance or systematically. Systematic errors or bias, usually take form through limitation of study design, confounding factors, and through collection of data [57]. In this section of the thesis we will address the internal validity, or to which length the data and observations found in our study are true. We will also address the extent to which our results can be generalized to other populations.

5.1 Study design

A cross sectional design is of an observational and descriptive nature, and it is representative of data collected from a subset of a population defined at a single point in time. The design is good to describe several variables simultaneously, find strong associations, and generate a hypothesis. [29,58] However, the study design also has some disadvantages, like the problem of causal interference. Since exposure to risk factors, and the manifestation or absence of disease is assembled at the same time, it is a study design that cannot provide evidence of causality [58] Further, the design is also unguarded against bias from high mortality diseases and rare exposure variables [57]. However, since mortality is irrelevant in the Fit Futures study, and the exposure variables are of ordinary nature, we assume for this reason that bias from the design is minimized. This study can also help provide suitable indications to if drinking sugar sweetened or carbonated beverages is associated with bone mineral density in adolescents.

5.2 Random error and precision

Some inconsistency of estimates is unavoidable since most predicted associations involve random errors. This can produce greater variability of the estimates, which can best be managed through greater precision; *"precision in measurement and estimation corresponds to the reduction of random error"* [57]p.29.

To minimize inter observer variability, the Fit Futures study was conducted in a specialized designed medical ward with experienced and trained personnel performing all collection of data. To prevent and reduce errors, the technicians worked according to detailed protocols developed by the administration before the study began [3].

Further, this study included a large sample size with over 400 participants of each sex, which decrease the effect of random errors. We can never exclude random errors, but we believe it's fair to say that this study is less vulnerable for random errors and statistical coincidences due to the large selection of participants.

5.3 Systematic Errors – Selection bias

Another treat to internal validity is selection bias. Selection bias is defined as *"a systematic error that results from procedures used to select subjects and from factors that influence study participation"*[59]*p.96.* The problem of selection bias often occurs when the association between exposure and outcome vary in the group of participants and the individuals who did not respond to attend the survey. This can produce distorted results compared to if you tested the true measure of the entire target population. In the Fit Future study, we only have a selection of adolescents who attend school. However, over 90 % of adolescents from 16-18 years in Norway are attending upper secondary school, so we consider this a strong base to say something about the general population in this age group. [60]

In 2012 when Fit Future 1 was conducted, 70 of the enrolled students for first year upper secondary school were missing, probably because they quit before school started. 114 of the students were unable to get a hold of for unknown reasons, or had persistent disease making them unable to attend school. 7% of the students invited choose not to participate in the study. [29] The group of non-attending adolescents could have influenced the outcome in various ways. Quitting school could be linked to unhealthy lifestyle, and chronic or long lasting disease could be associated with lower BMD[57].This could lead to a misrepresentation of high BMD levels of the participants in Fit Futures, compared to the population. However, since we have data from 93 % of the study population, the non-attending adolescents will probably produce systematical errors of little influence. We therefore believe that selection bias will not distort the result.

5.4 Information bias

Internal validity can also be threatened by information bias. Information bias can arise due too systematically imprecise or incorrect measurements of exposure or outcome variable, or if participants in a study report incorrect information.[57] Frequent reasons for this type of bias are incomplete definitions of study variables, imprecise procedures for data collection, or measurement and misclassification errors. In regards to systematical information bias, there are two types of misclassifications (measurement error); differential and non-differential. [57] Non-differential is less critical, and refers to when the error is independent of the exposure and outcome. This means that all study subjects or variables have the same probability of being misclassified. Differential error is more serious since the probability of error is different across study subjects and can change the result in unforeseeable directions. It is important to try and minimize differential error in the planning phase of a study.

5.4.1 Validity of bone mineral measurements

Dual x-ray absorptiometry (DXA) is a simple and quick x-ray technology used to measure body composition. It is considered the golden standard in measuring bone mineral density at all ages. In regards to validity, DXA is a technology that have good precision, advantages of short scan time, easy patient set up, and effective standardized procedures to ensure high quality. [61] [62]

In FF, Bone Mineral Density of the total body was our main outcome. All scans were performed on the same device by specially trained personnel at UNN, following a manufactured protocol to help minimize measurement errors. The coefficient of variation (CV) for the device used in FF is estimated to be 1.17 % at the femoral neck, and 1.2 % at the total hip [3]. CV for the total body has not been calculated. 10 scans were excluded due to quality control and unforeseen circumstances.

There also have some limitations, and one of the problems is that DXA is a two-dimensional projection measurement. This means that the measurement does not reflect the true volumetric bone density[57]. Usually the measurement error is underestimation of small bones and overestimation of larger bones. To manage this problem, the software from the manufacturer describe the BMD measurement in relation to age, gender and race [3]. The Z-score was calculated based on Lunar DXA pediatric application, version 1.34.

Sexual Maturity is also important to consider in understanding the estimates, since puberty is closely linked to height and growth. [29] To reduce systematical measurement errors, our multiple regression analysis was stratified by gender to exclude bone size imprecision related to sex. We also adjusted for age, BMI, and sexual maturity.

5.4.2 Validity of Sugar sweetened and carbonated beverages variables

In a review by Riordan and her colleagues [63], they investigated a range of instruments generally used to assess SSB intake in pan-European studies. Their review highlighted that there is a lack of standardization in self-administered assessment methods. One problem is that there are various definitions of SSB, and often no differentiation between soft drinks, diet soft drinks and squashes. The review also pointed out that frequency categories and portion size need to be assessed in a more systematic manner. The deviation in assessment methods can influence the comparability of intake data across studies.

The questionnaire used in Fit Futures has not been validated for adolescents and must therefore be interpreted with some caution. [34] In FF the adolescents were asked "How often do u usually have soft drinks with sugar (a ½ litre bottle equals 2 glasses)?", and consequently "How often do you usually have soft drinks with artificial sweetener?". They were also asked about squash with sugar and artificial sweetened squash. When creating the beverage variables, all variables were recoded to average number of glasses the participants would consume on a daily basis. Since this is only an approximation, there might be some errors in the quantity of this estimate, but the ranking should however be correct.

The definition of SSB is a broad term, and the consumption of sports drink, various energy drinks, lemonade and ice tea are some examples of beverages that also fall under this category. This was not specified in the questionnaire, and it is therefore not unlikely to think that these type of beverages are under reported. This makes the study susceptible to information bias. Recall bias is also always a risk when participants are asked remember events retrospectively by using self-reported instruments.

5.4.3 Validity of other confounding variables

BMI measurement is a commonly used tool to define body size and proportions in relation to height and weight. The collection of anthropometric data was performed by trained research personnel with equipment that was continuously adjusted and regulated. This will limit the concern of measurement error for the BMI variable. [29] However, the BMI measurement has been criticized for not reflecting a person's percent of body fat, [64] and this might lead to some misrepresentation of the relationship between BMI and BMD.

The golden standard for classification of sexual maturity are today considered to be the Tanner Stages. [65] Due to practical issues it was difficult to use this approach in Fit Futures.[29] Therefore, menarche age for girls and PDS questions for boys were used as data on puberty in this study. Menarche age is considered reliable and well validated measurement for pubertal development. [66] Findings in previous studies has shown that the age of menarche is a memorable and important moment, and therefore girls have good ability to recall the precise month and year of their menarche. [65]

The PDS questions on secondary sexual characteristics for boys was introduced later in the survey. PDS administered by self-reporting questionnaires is considered to be a reliable alternative to the Tanner stages. [65] It is regarded most reliable for longitudinal studies and cross sectional studies due to more broad and rough estimates. We considered the validity to be sufficient in this study when handled as a covariate.

The data on puberty, physical activity level, screen time, alcohol use, smoking, and dietary variables were all assembled by questionnaire. This can produce some problems in regards to misconception of categories and memory (recall bias). Former studies have shown that adults have a tendency to misreport things like nutritional information, smoking, and alcohol habits.

[67] [68] Variables like smoking and use of alcohol is often receptive to underreporting since they are linked to unfavorable health outcomes. [57]

Over or under reporting is also a concern among adolescents as they are exposed to social group pressures. It is not improbable that adolescents in this study will do the same. Self-reporting bias is hard to control for, but since the questionnaire was administered through a self-administrating software this could provide some better sense of neutrality and anonymity in regards to sensitive subjects. Further, the limitations of misconception and recall bias related to the questionnaire is likely to be non-differential, and will allegedly lead to underestimated associations. [57] The questionnaire must also be interpreted with some caution since it is not validated for adolescents in this age group.

In this study, the data on screen time (ScT) and physical activity (PA) are considered to be rough estimates. In regards to ScT, the data was based on hours spent in front of the television and in front of the computer. The questions did not go into depth about other sedentary activities or the use of different screen based activities [34].

The challenge when assessing PA is the individual variance in condition, which is often changing in line with different seasons, weeks, and days [57]. At the moment, objective measurements instruments (like heart rate monitoring etc.) combined with subjective measurements like self-reporting questionnaires and interviews is viewed as the most appropriate method [57]. When using a questionnaire, like in FF, this will present challenges related to recall bias, wrong interpretations, and all the different aspects related to PA especially. This could lead to wrong representation of the observed relationship between these variables and BMD, which may threat validity and reliability.

In regards to the questionnaire and the other various dietary variables, there was a problem that the questions were not adequately specified. One example is when the adolescents are asked "How often do you eat (all kinds) of cheese?". Specifications about the quantity (like, slices of cheese in this example) and type of food were not asked about. This will give more rough estimates of the adolescent's nutritional consumption.

5.5 Generalizability (external validity)

Internal validity is imperative to external validity, and external validity is related to if the findings in our study sample is representative to a larger population. In other words, if this study sample is generalizable to other adolescents in this age group. [34]

When a study has no self-selection or randomization, like the FF, the distribution of characteristics is more similar, and the generalizability goes up[34]. Since FF includes all first-year upper secondary school students, it would be fair to say that this study is highly representative for other groups that distribute corresponding characteristics.

Other studies have however reported higher prevalence of overweight among adolescents in the Nordic county of Norway, and also slightly higher levels of physical activity have been reported in this study compared to other adolescents in the Young-HUNT study. [34] Since BMI and physical activity are important predictors of BMD, there is a chance that these significant variables can contribute to higher BMD levels in our study sample compared to the general population of Norwegian adolescents. In regards to statistical generalization, the findings in this study may not be representative for all Norwegian adolescents at this age.

6. Discussion of the Findings

While conventional strategies have been focusing on decreasing age-related bone loss and fractures, the new strategies have in the recent years directed its attention towards the acquisition of peak bone mass (PBM), and how early life experiences can influence our physiology and genetic constitution for optimizing good bone health later on in life.

Former research has suggested that consumption of carbonated soft drinks during adolescence can influence bone mineral accrual and PBM, and thereby increase fracture risk later on. This is of concern since the consumption of soft drinks has increased substantially in the past decade, and Norway is one of the leading country in regards to high consumption of SSB. Carbonation and exaggerated intake of some substances, like sugar, caffeine, and phosphoric acid found in these beverages have also been linked to bone resorption. The aim of this study was therefore to investigate if moderate or high consumption of SSB or CB is associated with bone mineral density in adolescents. After adjustment of relevant confounding variables, no significant association between consumption of SSB or CB and BMD was found. Only for girls who reported to never drink SSB was there found an association to bone mass, with higher BMD values (0.016 g/cm^2) for total body compared to girls who drank half a glass daily. A significant association was also found in girls reporting to never drink artificial sweetened beverages, only here the BMD values were lower (-0.016 g/cm^2) compared to the reference group. These finding are similar to other studies who have reported an association between carbonated soft drinks and BMD only in girls [20] [18] [24] [21]. This suggests that gender differences during puberty can make girls more susceptible to the adverse effects that carbonated soft drinks may have on BMD.

In regards to the confounding variables adjusted for in our analysis, the highest levels of physical activity and BMI were in general the most important predictors to higher BMD

values for both sexes. For boys, drinking alcohol twice or more per month also had positive association to bone mass. The protective effect that body mass, exercise, and moderate consumption of alcohol can have on bone have also been reported in previous studies [29] [32,57] [37].

For girls, late menarche was associated with lower BMD levels, but for boys we observed a change in sexual maturity category "barely Started" in the univariate analysis to the main analysis. In the univariate analysis boys who reported to develop late had lower BMD levels (-0.043 g/cm^2) compared to the reference group However, in the main analysis higher BMD values (0.060 g/cm) was observed in this same category. This suggests that even though late puberty in itself is associated with lower BMD, the interaction of other confounding variables adjusted for in this analysis can change this observed relationship in boys.

The findings in this present study is consistent with several previous studies, where no association between the total consumption of carbonated or sugar sweetened beverages and BMD has been found [69] [15,70]. Several studies have however found an association to cola drinks, but no association to other types of carbonated soft drink beverages [26] [18]. However, the relationship of carbonated soft drink consumption and BMD is still not very clear. Few studies have to our knowledge confirmed that soft drink consumption is associated to BMD as long as calcium level is maintained at a sufficient level [20,24] [21] [71] [25].

Reports in previous studies suggests that displacement of milk is an important component that mediates the effects of bone resorption caused by soft drinks. [21] [24]This is because dietary calcium through milk consumption can help balance the concentration of phosphorus. If there in an inadequate intake of calcium, this can cause elevated PTH secretion, and thereby changes in the calcium phosphorus ratio leading to increased bone turnover. [71]

In a 8-week metabolic study [69] they investigated if nonalcoholic carbonated beverages could influence the calcium serum or urinary markers of the calcium metabolism in eight healthy women who received either a control diet or a diet high on non-alcoholic carbonated soft drinks. They concluded that there seemed to be no adverse effect from drinking non-alcoholic carbonated beverages on the calcium metabolism or the calcium urinary excretion. [69] This also gives support to the conclusion that soft drinks relationship to calcium deficiency is most likely due to the replacement of milk. In our study, as much as 74 % of the girls and 76 % of the boys reported to drink milk daily, which possibly could have been a mediating factor in balancing the calcium phosphorus ratio in our study population. However, our study did not investigate if carbonated or sugar sweetened beverages is associated to bone turnover when these beverages are a replacement for milk in the diet. Further, dietary calcium was not adjusted for in our analysis. The variable daily milk consumption was eliminated in the preliminary analysis due to no association with BMD total body.

Several studies have also suggested a relationship between cola drinks and BMD in adolescents. In a population based case-control study [70] they looked at the association between soft drink beverages, consumption of milk, physical activity, bone mass, and upper limb fractures in 9-16 years' old children and adolescents. Data was collected retrospectively through a questionnaire were information regarding lifestyle (exercise and screen time) and consumption of milk and various CB. Measurement on BMD of the spine, hip and total body was attained through DXA. They concluded from the study that cola drinks are associated with increased risk of wrist and forearm fractures in the adolescents. This effect was mediated by watching television and BMD, but not by reduced intake of milk. No association between the total intake of CB and fractures was observed.

In a study by Tucker et al. (2006) they looked at the association of soft drink consumption in relation to BMD at the spine and hip in 1413 women and 1125 men by using DXA scans and food-frequency questionnaires. In a regression analysis they also adjusted for other confounding variables like BMI, height, age, energy intake, physical activity, substance use, intake of calcium and vitamin D, caffeine from non-cola sources, estrogen, and menopausal status. They found that consumption of Cola had adverse effects on bone mineral density at the hip, but not the spine. The result was only significant for women, and there was found no significant relationship to non-cola carbonated beverages. This suggests that caffeine content, and not the phosphoric acid or the sugar content in soft drink beverages can contribute to a lower BMD. [26]

In the present study we had no information about if the carbonated or sugar sweetened beverages contained any caffeine. And since we only looked at BMD total body as our outcome variable, we can't say anything about the association between soft drink beverages and other bone sites in adolescence.

The results from previous studies are conflicting, but there seem to be a certain consensus that carbonated or sugar sweetened beverages are not associated with BMD as long as calcium intake is at a sufficient level. The effect of dietary calcium seems to act as a protective mechanism against the adverse effects of soft drinks can have on bone. However, several studies have reported that the caffeine content in Cola drinks can contribute to lower levels of BMD. There also seem to be a gender difference, where harmful effects of soft drinks are more prone to women. This could be explained a difference in interaction of hormones between the sexes, or that women are more sensitive to nutrient deficiency.[26] Women also have smaller bone mass compared to men. Others have suggested that since men usually have a higher levels of physical activity and consume more calcium than women, they are

protected against adverse effect of soft drink beverages.[26] However due to limitations in the study design in former studies, additional research is needed to confirm these findings.

6.1 Conclusion

In the present study we found no association between consumption of sugar sweetened or carbonated beverages when adjusted for confounding variables. The strength of this study is high attendance rate of both genders, with a representation of both urban and rural adolescents. However, with a cross sectional design, we cannot provide evidence of causality. Further, limitations in regards to the nature of our questionnaire can make the study vulnerable to (non-differential) information bias. Since the questionnaire is not validated for adolescents, these results must be interpreted with some caution. Another limitation in this study is that vi had no adjustment for calcium.

The problem of when assessing the consumption of soft drinks in relation to bone health is the lack of standardization in self-administered assessment methods. Various definitions of the term soft drinks or SSB, and lack of differentiation between types of soft drinks and frequency can make it difficult to compare our result to other studies. In the present study it is not unlikely that sports drinks, energy drinks, lemonade etc. are underreported due to lack of specification in our questionnaire.

There are several aspects of this subject that needs further research. One aspect is the relationship between soft drinks, BMD, and gender differences. Further, the role of phosphorus and caffeine content, which was not addressed in this study, also need to be studied more extensively. We hope that this study can give implications for further research.

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MILJØGIFTER

ASTMA OG ALLERGI

HVA ER FIT FUTURES?

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

HVEM KAN DELTA?

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

Vi ønsker både nye og tidligere deltakere velkommen!

HVORFOR ER DETTE VIKTIG?

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

HVA FORSKES DET PÅ?

Hovedområdene det forskes på er:

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

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

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

SLIK FOREGÅR UNDERSØKELSEN

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

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

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

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

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

MULIGE ULEMPER OG FORDELER

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

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

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

TIPS OG RÅD FØR UNDERSØKELSEN

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

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

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

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

INFORMASJON FRA ANDRE KILDER OG BRUK AV DATA I FRAMTIDEN

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

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

STØY

TANNHELSE

FRIVILLIG DELTAKELSE

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

HVA SKJER MED DE BIOLOGISKE PRØVENE?

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

PERSONVERN OG SIKKERHET

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

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

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

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

RETT TIL INNSYN, SLETTING AV PRØVER OG OPPLYSNINGER

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

BRUS & JUICE

VIL DU DELTA?

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

ANSVARLIGE FOR GJENNOMFØRING AV FIT FUTURES

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

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

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

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

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

SPØRSMÅL?

Dersom du har spørsmål om undersøkelsen, kontakt:

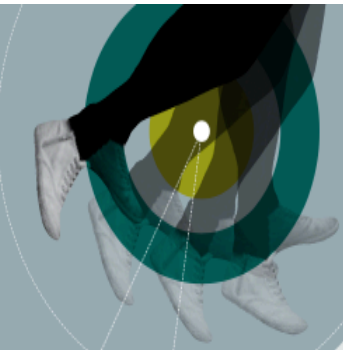
- Prosjektadministrator **Annelene Moberg** på telefon 93 00 39 25
- Prosjektadministrator **Siv Normann Gundersen** på telefon 93 00 39 54
- **Forskningsposten UNN** på telefon 77 62 69 09

WWW.FITFUTURES.NO

UNN

UNIVERSITETET I TROMSØ

folkehelseinstituttet



AKTIVITET

2012
2013

ENERGI

FitFutures 2
EN DEL AV TROMSØUNDERSØKELSEN

DIN HELSE DIN FREMTID

INVITASJON TIL Å DELTA I HELSEUNDERSØKELSE BLANT UNGDOM

Appendix 2

VIL DU DELTA?

Samtykke til å delta i studien Fit Futures 2

Jeg er villig til å delta i studien

_____

(DITT FULLE NAVN I BLOKKBOKSTAVER)

Sted _____

Dato _____

_____

(DIN SIGNATUR)

Appendix 3

Link til Fit Futures spørreskjema gjennom questback:

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