Associations of sedentary behavior, physical activity, body composition, sleep and vitamin D with bone stiffness

Cross-sectional and longitudinal findings from the IDEFICS and I.Family cohort

Dissertation

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Hiermit erkläre ich, Lan Cheng, geboren am 12.07.1992, dass für das Verfassen der vorliegenden Dissertation “Associations of sedentary behavior, physical activity, body composition, sleep and vitamin D with bone stiffness: Cross-sectional and longitudinal findings from the IDEFICS and I.Family cohort” folgende drei Aussagen zutreffen:

1. Ich habe die Arbeit ohne unerlaubte fremde Hilfe angefertigt.
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Bremen, August 2021
Lan Cheng

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Abbreviations

aBMD	areal bone mineral density
BMC	bone mineral content
BMI	body mass index
BUA	broadband ultrasound attenuation
CTx	C-terminal telopeptides of type I collagen
DXA	dual energy X-ray absorptiometry
GH	growth hormone
IGF-I	Insulin-Like Growth Factor-I
MVPA	moderate-to-vigorous physical activity
1,25(OH)D	1,25-dihydroxyvitamin D
PTH	parathyroid hormone
QCT	quantitative computed tomography
QUS	quantitative ultrasound
SOS	speed of sound
25(OH)D	25-hydroxyvitamin D
vBMD	volumetric bone mineral density

Outline of the thesis

The present thesis is a cumulative dissertation with five main chapters. The introduction chapter (*Chapter 1*) gives an overview on childhood bone health from basic bone biology to epidemiological findings. *Chapter 2* describes the research hypotheses and aims of the present thesis. *Chapter 3* presents the study population, measurement methods of bone health outcomes and determinants as well as applied statistical strategies. *Chapter 4* summarizes the main findings and provides a general discussion on the results, strengths and limitations. The conclusion chapter (*Chapter 5*) provides the public health implications of the present work and future research directions. Four original peer-reviewed publications/submitted manuscripts forming this thesis are listed below and also attached in the Appendix.

1. Cheng L, Pohlabeln H, Ahrens W, Russo P, Veidebaum T, Chadjigeorgiou C, Molnár D, Eiben G, De Henauw S, Moreno L, Page A, Hebestreit A; IDEFICS and I.Family Consortia. Sex differences in the longitudinal associations between body composition and bone stiffness index in European children and adolescents. Bone, 2020, 131: 115162.

2. Cheng L, Pohlabeln H, Ahrens W, Lauria F, Veidebaum T, Chadjigeorgiou C, Molnár D, Eiben G, Michels N, Moreno LA, Page AS, Pitsiladis Y, Hebestreit A; IDEFICS and I. Family Consortia. Cross-sectional and longitudinal associations between physical activity, sedentary behaviour and bone stiffness index across weight status in European children and adolescents. Int J Behav Nutr Phys Act, 2020, 17(1): 1-13.

3. Cheng L, Pohlabeln H, Ahrens W, Russo P, Veidebaum T, Hadjigeorgiou C, Molnár D, Hunsberger M, De Henauw S, Moreno LA, Hebestreit A; IDEFICS and I.Family consortia. Cross-sectional and longitudinal associations between sleep duration, sleep quality, and bone stiffness in European children and adolescents. Osteoporos Int , 2021, 32(5): 853-863.

4. Cheng L, Pohlabeln H, Wolters M, Ahrens W, Siani A, Veidebaum T, Tornaritis M, Molnár D, Eiben G, Hunsberger M, De Henauw S, Moreno LA, Hebestreit A, IDEFICS and I.Family consortia. Associations between serum 25-hydroxyvitamin D status, bone turnover markers and bone stiffness in European children and adolescents. Submitted to Am J Clin Nutr.

Summary

In children and adolescents, bone modeling and remodeling is highly active in order to expand bone in length and width, to increase bone mass, and to maintain bone shape. Although bone mass acquisition is relatively slow throughout childhood, with the onset of puberty and the growth spurt of height in adolescence the rate of bone mineral accumulation increases, reaching a peak bone mass shortly after a peak height. The peak bone mass is an important predictive factor of osteoporosis in the later life due to the bone loss during ageing. Except for genetic factors, there is an estimated 20 to 40% of the peak bone mass variation contributed to modifiable factors e.g., mechanical loading, physical activity, sedentary behavior, sleep and nutritional factors. However, we are only beginning to identify the specific dimensions and doses of these modifiable factors needed for the short-term and long-term beneficial effects on bone health. The lack of longitudinal epidemiological studies and the conflicting results in the intervention studies among healthy pediatric populations limit our knowledge. Hence, the present thesis aims to provide a better understanding on the associations between physical activity, sedentary behavior, sleep, nutrition and bone health in children and adolescents.

The present thesis is based on the data from the IDEFICS/I.Family cohort including children and adolescents aged 2 to 15 years from eight European countries. Three examination waves with repeated measurements were conducted in 2007/2008, 2009/2010 and 2013/2014. In the subgroups, bone stiffness index was measured using calcaneal quantitative ultrasound (QUS) in all examination waves, serum bone formation marker osteocalcin was analyzed using chemiluminescence assays in the first examination wave while serum bone resorption marker C-terminal telopeptides of type I collagen was analyzed in the first and third examination waves. Calcaneal QUS as a validated method to estimate bone health is becoming popular in pediatric populations since it is non-radiating, quick and cost-effective. The measured parameters of broadband ultrasound attenuation and speed of sound, as well as the derived stiffness index are related to bone mass and bone structural properties. Meanwhile, bone resorption and formation markers have been suggested to be sensitive to the changes in environmental factors, hormone levels and treatments. Therefore, stiffness index, osteocalcin and C-terminal telopeptides of type I collagen were considered as bone health outcomes.

Body composition in terms of fat mass and fat free mass was derived from objectively measured skinfold thickness. Weight status was estimated from objectively measured body height and weight by calculating body mass index z-scores and cut-offs. Physical activity and sedentary behavior were measured using both self-administrated questionnaires and accelerometers. Sleep duration and quality were evaluated using questionnaires. Consumption frequency of dairy products and usual calcium intake were collected using food frequency questionnaires and 24h-dietary recalls, respectively. Serum 25-hydroxyvitamin D was analyzed using chemiluminescence assays. Linear mixed-effect models were used with adjustments for a cluster effect of country and potential confounders. The major findings were presented and discussed in four published or submitted original papers, final sample sizes that varied in each paper depended on the analysis strategies for different research questions.

First, the longitudinal results indicated a positive relationship between fat free mass and stiffness index during growth. Specifically, baseline fat free mass was observed to predict two-year and six-year changes in stiffness index, and six-year changes in fat free mass was also positively associated with change in stiffness index. Meanwhile, the association between six-year changes in fat mass and stiffness index differed by sex and pubertal status, suggesting an inverse association in boys and girls before menarche, but a positive association in girls after menarche *(Lan Cheng, et al., Bone. 2019)*.

Second, objectively measured moderate-to-vigorous physical activity (MVPA) was positively associated with the increase of stiffness index over two years and six years of follow-up. These results were supported by the comparable albeit weaker positive associations between self-reported time spent at sports clubs and stiffness index. However, the inverse associations between screen time as a surrogate for sedentary behavior and stiffness index depended on weight status. Specifically, the cross-sectional association between weekly duration of watching TV and stiffness index was observed to be inverse only in thin/normal weight group. Both baseline and two years change in weekly duration of watching TV, and six years change in weekly duration of playing computer/games were inversely associated with corresponding changes in stiffness index in the overweight/obese group *(Lan Cheng, et al., Int J Behav Nutr Phys Act. 2020)*.

Third, the association between sleep and stiffness index was analyzed using data from two follow-up examination waves with the interval of approximately four years. Total sleep duration was calculated and further classified into short, adequate and long based on the recommendation from the National Sleep Foundation. Poor sleep quality was estimated by reporting either having trouble to get up in the morning, or having difficulty to fall asleep, or have no regular bedtime routine. The positively cross-sectional associations between nocturnal sleep duration, daytime napping and stiffness index were only observed in participants with adequate sleep duration. After four years of follow-up, the positive association between daytime napping and stiffness index was more pronounced in participants with short sleep duration. Moreover, long-term detrimental effect of extreme sleep duration (short or long) on stiffness index only existed in participants with poor sleep quality *(Lan Cheng, et al., Osteo Int. 2020)*.

At last, only cross-sectional analyses were conducted in the associations between vitamin D, bone turnover markers and stiffness index using merged datasets based on the first and third examination waves. Serum 25-hydroxyvitamin D, calcium intake and dairy products consumption were observed to be inversely associated with bone resorption marker but not formation marker. MVPA modified the association between 25-hydroxyvitamin D and stiffness index, suggesting that serum 25-hydroxyvitamin D no less than 20 ng/ml would be a protective factor for calcaneal stiffness index only if children met the MVPA guideline of one hour MVPA per day on average *(Lan Cheng, et al., Am J Clin Nutr. submitted)*.

In summary, the present cumulative thesis provides a comprehensive understanding on the associations between lifestyle-related factors and bone health indicators in children and adolescents. Future prevention and intervention studies with regard to improving childhood bone health should put emphasis on promoting sufficient MVPA, maintaining adequate sleep duration and calcium intake, and improving consumption frequency of dairy products. Some bone health determinants in specific groups i.e. excess fat mass in boys and pre-pubertal girls, long screen time in overweight/obese children, and insufficient vitamin D level in inactive children particularly need attention.

Content

1 Introduction

1.1 Bone anatomy and histology

Bone is the dynamic and complex organ in the skeletal system; it is characterized by its rigidity, flexibility and strength, as well as power of growth, repair and regeneration (1, 2). In the human, there are approximately 270 bones at birth, but many of these merge together during development, leaving a total of 206 individual bones in the adult (3). The bones not only support the body, facilitate movement, and protect the vital organs, but also serve as multiple important roles e.g., environment for bone marrow, mineral storage for calcium and phosphorus homeostasis, reservoir of growth factors and cytokines, and taking part in acid-base balance (1).

Bones have a variety of shapes and sizes as well as complex external and internal structures. There are five types of bones classified by their shapes: long, short, flat, irregular, and sesamoid. At the macroscopic level, the external surface of the bone is covered with a fibrous membrane called the **periosteum**, which contains rich blood vessels, lymphatic vessels and nerves. The deep or cambium layer of periosteum is populated with highly osteogenic cells contributing to bone metabolism, growth and repair. Bone tissues can be divided into two types: **cortical bone** and **trabecular bone**, their distribution and concentration vary depending on specific regions. In general, **cortical bone, which is also called compact bone**, is under the periosteum and forms the dense and hard outer layer of bones. It can withstand compressive forces, and comprises 80% of bone mass of an adult human skeleton. **Trabecular bone, which is also called cancellous bone or spongy bone**, is the interior tissue or found at the ends of long bones and near joints with an open cell porous network called trabeculae. It can support load by redirecting stresses, and comprises the remaining 20% of bone mass but has nearly ten times the surface area compared to cortical bone. Trabecular bone is embedded with a connective tissue membrane called the **endosteum**, which is a discontinuous layer of osteogenic cells, and often contains red bone marrow where hematopoiesis occurs. It is also important for the regulation of calcium exchange between bone and the extracellular fluid compartment (1-3).

At the microstructural level, bone tissue is mainly composed of bone matrix with entrenching a relatively small number of bone cells. Bone matrix consists of inorganic and organic components. The inorganic portion is mainly made up of calcium and phosphate in the form of hydroxyapatite crystal, giving bones their stiffness, hardness and strength. The organic portion mainly consists of cross-linked type I collagen to give bones their resilience, ductility and flexibility so that they do not become brittle. There are four types of cells within bone tissues: osteogenic cells, osteocytes, osteoblasts, and osteoclasts. **Osteogenic cells** (stem cell) from the deep layer of the periosteum, endosteum and bone marrow can differentiate into mature and active osteoblasts, which are responsible for bone formation. **Osteoblasts** produce bone matrix by synthesizing the collagen fibers and facilitating the mineral deposition. After secreting enough matrixes surrounding the osteoblasts within the ossification centers, some of them change into **osteocytes**. Osteocytes are the most abundant cells in the bone tissue; they can induce endocrine processes in response to mechanical and hormonal changes in bone. **Osteoclasts** originating from white blood cells are responsible for bone resorption. Even though these bone cells only compose a small amount of the bone volume, they are crucial to the process of bone modeling and remodeling (4, 5).

1.2 Bone growth, modeling and remodeling

Skeletal development begins during the embryonic stage and continues into the late second or early third decade of life until bone maturation (6). There are two distinct processes in the bone development: intramembranous ossification and endochondral ossification. These modes of development are differentiated depending on which ossification is initiated and which cells produce the matrix. Briefly, the process of **intramembranous ossification** refers to when osteogenic cells differentiate directly into osteoblasts to produce bone matrix. This process mainly occurs during fetal development and results in the formation of flat bones. **Endochondral ossification** is the process when osteogenic cells differentiate into chondroblasts to form chondrocytes and hyaline cartilage templates. The membrane that covers the cartilage (perichondrium) is replaced by periosteum over time, and the cartilage is also gradually replaced by mineralized bone tissue after the death of chondrocytes (7, 8).

Bone modeling is the most prominent activity during growth, it primarily serves to expand bone in length and diameters, to increase bone mass, and to maintain bone shape (9). For a typical long bone in growing children, it consists of a diaphysis or shaft forming the long axis, epiphyses at two ends, and an epiphyseal plate (or growth plate) in between them. The longitudinal growth is dependent on the epiphyseal plate via endochondral ossification: the cartilage is formed on the epiphyseal side while the cartilage is ossified on the diaphyseal side. The continuous formation and ossification promote the diaphysis grow in length, and this process lasts until early adulthood (approximately 18 to 21 years old) when the epiphyseal plate becomes ossified as an epiphyseal line (10). Simultaneously, the radial growth of bones undergoes: osteoclast-mediated bone resorption lines the marrow cavity and establishes cortical and trabecular architecture, while osteoblast-mediated bone formation beneath the periosteum makes the bone thicker via intramembranous ossification. The continuous bone building and breaking down increase the diameter of both diaphysis and marrow cavity, and this process can continue even after longitudinal growth ceases (2, 3, 7, 8). The rate of bone modeling is highest during growth and then slows down during adulthood.

Bone remodeling is initiated by the activity of osteoblasts and osteoclasts to replace old bone matrix, repair small defects and renew skeleton, resulting in maintaining or slightly decreasing bone mass (9). In contrast to bone modeling with bone matrix resorbed on one surface and deposited on another, resorption and formation of remodeling occur on the same surface. The rate of bone remodeling is very high during growth and then slowly decreases until peak bone mass is attained. In adulthood, 5% to 10% of the skeleton is remodeled annually even without injury or exercise. Each complete remodeling cycle from the time of osteoclast-mediated bone resorption to the time that osteoblasts finish producing bone matrix takes about four to six months (2, 7).

1.3 Bone health assessment in pediatrics

The concept of bone health is generally defined by bone strength and metabolism (11). The strength of the bone depends on bone mass, bone geometry and architecture, which are normally measured by densitometry techniques e.g., the dual energy X-ray absorptiometry

(DXA), quantitative computed tomography (QCT) and quantitative ultrasound (QUS) (12). The biochemical markers of bone formation and resorption representing bone metabolism can be measured in the blood and urine (13). These measurement techniques capture different aspects of bone health and have their own strengths and limitations.

Clinically, **DXA** is the most recommended tool to monitor bone health in childhood because of its speed, low-ionizing radiation dose, and robust pediatric reference data. As a 2-dimensional densitometry technique, it primarily measures **areal bone mineral density** (aBMD, g/cm^2), **bone mineral content** (BMC, g) and bone area (cm^2) (14). According to the 2013 Pediatric Position Development Conference of the International Society for Clinical Densitometry, the preferred skeletal sites for DXA assessment in children are the lumbar spine and total body less head, while the assessment at other skeletal sites e.g., the forearm, the femoral neck, the proximal and distal femur should be carefully interpreted for monitoring and fracture prediction (15). Nevertheless, DXA measurements are size-dependent, which means that even if volumetric bone density is identical in children, the value of aBMD will be lower in children with smaller bones while higher in children with larger bones (14, 16). Besides, DXA-derived aBMD only provides integrated measures of cortical and trabecular bone, and it only explains about 60 to 70% of the variance in bone strength due to the lack of structural information (17).

QCT as a newer 3-dimensional densitometry technique is primarily used for research rather than clinical conditions. Compared to DXA-derived aBMD, it not only provides a true volumetric BMD (vBMD, mg/cm^3), which is less influenced by growth, but also separately evaluates the trabecular and cortical compartments (14). Moreover, it can capture bone parameters of geometry (e.g., cross-sectional area of bone, cortical and trabecular thickness, periosteal and endosteal circumference) and/or microarchitecture (e.g., bone volume ratio, trabecular number and cortical porosity), those can be used to calculate estimates of biomechanical bone strength (14, 18). There are mainly three types of QCT devices: **central QCT** is used for whole body scan, the most commonly scanned sites are the lumbar spine (L1 to L3), mid femoral shaft and the tibia; **peripheral QCT** is only applied to peripheral skeletal sites such as the radius, the tibia and mid femur, and these measurements are obtained at much

lower cost and radiation exposure than central QCT; **high-resolution peripheral QCT** has higher spatial resolution compared to standard peripheral QCT, which additionally allows it to assess the microarchitecture, and to accurately evaluate bone strength (19). In spite of the advantages of QCT devices, the availability is problematic in healthy pediatric population because of their high cost, ionizing radiation dose, and the paucity of reference data.

QUS measurements are based on the ultrasound wave passing through the specified region of interest: the speed of sound (SOS, m/s) represents the velocity of sound traveling through the bone; the broadband ultrasound attenuation (BUA, dB/MHz) represents the energy of sound absorbed by the bone; and the bone stiffness index is calculated from the SOS and BUA based on the equation of stiffness index = (0.67 * BUA) + (0.28 * SOS) - 420 (20). It has been suggested in vitro and vivo studies that QUS parameters are related to BMD, bone microarchitecture and mechanical parameters, with the reduced values indicating lower bone stiffness and higher fracture risk (21). QUS devices are mainly applied on peripheral skeleton sites such as the calcaneus, the radius, the phalanges, the patella and the tibia. However, the calcaneus is the only recommended measured site for clinical applications with regard to fracture risk assessment according to the 2007 Pediatric Position Development Conference of the International Society for Clinical Densitometry (22). The calcaneal bone consists of 90 % trabecular bone, which is generally more metabolically active than cortical bone and thus more sensitive to the changes in BMD. Moreover, the calcaneus has little surrounding soft tissue, and also reflects the mechanical loading of body weight. In children and adolescents, the comparison of calcaneal QUS-derived stiffness index with DXA-derived aBMD at the calcaneus, hip or spine reported strong correlations of 0.4 to 0.7 (23-26). Besides, QUS devices are cost-effective, portable and non-radiating; these characteristics make them more and more popular in large-scale epidemiological studies particularly among pediatric populations. However, because of the poor knowledge on specific bone properties reflected by QUS devices, their application in clinic and research remains controversial (21).

Biochemical markers of bone turnover are enzymes and non-enzymatic peptides from cellular activities of osteoblasts and osteoclasts. Practically, bone formation markers include products secreted by osteoblasts during bone matrix formation (e.g., bone-specific alkaline phosphatase

and osteocalcin) and fragments of collagen cleaved during collagen synthesis (e.g., amino- and carboxy-terminal propeptide of procollagen type I). Bone resorption markers include mature collagen fragments released from the bone matrix (e.g., C- and N-terminal cross-linked telopeptides of type I collagen) and the enzyme secreted by osteoclasts (e.g., tartrate-resistant acid phosphatase type 5b isoform) during resorption (27). In adults, bone turnover markers reflecting bone remodeling have been reported as independent risk factors for osteoporosis and fracture (28). However, clinical significance of bone turnover markers in children and adolescents is controversial, since they simultaneously represent not only homeostatic remodeling but also modeling and linear growth of the skeleton (29). Besides, evidence regarding the relationship between bone turnover markers and bone physical properties with regard to bone mass and architecture also remains unclear. Generally, consistent but relatively weak inverse correlations have been reported between bone turnover markers and DXA-derived bone mass, and these correlations were observed at different skeletal sites, sex groups and pubertal stages with varied bone turnover markers (30-32). However, some longitudinal studies observed contradicting results, suggesting serum levels of bone turnover markers positively predicted subsequent DXA-derived aBMD or BMC at follow-up (33-35). Few studies investigated the relationship between bone turnover markers and CT-derived bone structural parameters: a two-year treatment study in children with secondary osteoporosis observed reduced level of osteocalcin and alkaline phosphatase along with increased second metacarpal cortical thickness and improved vertebral morphometry (36). Another cross-sectional study suggested that bone formation markers were inversely related to the material density of bone, while bone resorption markers were inversely related to the volume of bone (37).

1.4 Growth trajectories and reference values of bone health indicators

Skeletal growth is mainly modulated by genetic factors and follows age-, sex- and race-specific patterns (6, 38, 39). Evidence from studies in twins and families shows an estimated 60% to 80% of the variability in peak bone mass attributes to heritability, while the remaining 20% to 40% is influenced by hormone levels and environmental factors (40, 41). The process of skeletal development is uneven across the life span with three critical periods

of accelerated growth during fetal, infancy and puberty (42). Even though bone mass acquisition is relatively slow during childhood, numerous prospective studies have demonstrated that aBMD tracks strongly from childhood through adolescence, with correlations ranging from 0.5 to 0.9 depending on different skeletal sites and follow-up durations (43, 44). After the height, bone mass and other skeletal dimensions achieve the adult size, and the skeleton continues to change over the subsequent decades with an inexorable age-related loss of both cortical and cancellous bone (45). Hence, optimizing bone health in childhood and adolescence is critical for preventing osteoporosis fracture in later life.

The amount of bone mineral acquired and the skeletal growth in the longitudinal axis from birth to adulthood are highly coordinated (39). Both of them predominate over the first two decades of life, but they do not occur at the same pace. Evidence from a longitudinal study suggests that children at age 7 years had obtained 69.5% to 74.5% of maximal observed height but only 29.6% to 38.1% of maximal observed whole body BMC (39). With the onset of puberty and the growth spurt in height, bone mineral accretion accelerates and reaches a peak after the closure of epiphyseal growth plates. It has been suggested that the peak height velocity occurs at approximately 13 years in boys and 11 years in girls, preceding the peak acquisition rates of whole body BMC by about seven months (39, 46, 47). Over the whole adolescent period, nearly half of peak bone mass is acquired. Another 7% to 11% of adult total BMC is accrued after linear growth ceases (39). The bone area plateaus one to two years earlier than BMC, peak bone mass occurs between the end of the second and the early of the third decade of life depending on skeletal sites and sex (47, 48).

It should be noted that peak bone mass is a broad definition for peak bone strength. Together with bone material properties (e.g., density), geometric and structural properties (e.g., size, thickness and architecture) also significantly contribute to bone strength. Evidence from studies using QCT suggested that the vertebral vBMD of trabecular bone is stable and comparable in boys and girls during pre- and early puberty, and the distinct increases occur between the ages of 10 to 15 years for girls and 12 to 17 years for boys (49). However, the trabecular structural parameters in long bone i.e., radius and tibia are relatively stable in girls throughout puberty, but significantly increase in boys from 15 years old (50). The vBMD of

cortical bone at radius and tibia increases more rapidly after epiphyseal closure and continues into the third decade of life (45). However, the cortical bone may go through a transient period of increased porosity between peri-puberty and post-puberty, resulting in decreased bone strength particularly for boys (50, 51). In general, total bone strength and cross-sectional area increases throughout growth and is larger in males than females of similar stature at all ages, even at birth (52).

Calcaneal QUS parameters as the comprehensive estimates for bone density and structural properties, their growth curves and reference values have also been reported in pediatric populations. A longitudinal study conducted in Caucasian children aged 7 to 18 years found that the most rapid growth in calcaneal BUA and SOS for boys was in early and late adolescence, while a constant linear growth for girls was observed throughout childhood. Nevertheless, there is no sex difference in the values of BUA and SOS measures within any age group (53). Most of cross-sectional studies calculated the reference values for children aged 6 years and above with reported positive associations between QUS parameters and chronological age. The sex differences were commonly observed in the age range of 11 to 13 years and 16 to 19 years with higher values in boys (54) or in girls (55, 56). In the IDEFICS study, the reference values of stiffness index for 2 to 10 years old healthy children were calculated by Hermann et al (57). In accordance with reported pediatric reference data, stiffness index was observed to be positively associated with age and height in children aged 6 to 10 years. However, inverse associations were observed in children aged 2 to 5 years. Comparable statistical method was used to calculate percentile curves for pooled IDEFICS/I.Family data. In the updated percentile curves, the stiffness index was positively associated with age and height in all age groups.

The changes in bone turnover markers coincide with the skeletal maturity. They are also strongly influenced by sex, age, and sexual development (58). Even though absolute values differ between studies, similar patterns have been observed for different biochemical markers. In general, the levels of bone turnover markers in newborn children increase rapidly until attaining the peak around the third month (29). Between the third month and third year of life, markers of formation and resorption decrease, and then remain constant or slightly increase.

This corresponds to the slowing of linear growth until puberty. In the IDEFCIS study, sex-, age- and height-specific reference values of serum cross-linked carboxy-terminal telopeptide of type I collagen (CTx) were calculated for 3 to 9 year-old children. The CTx values showed a linear-positive association with age and height, and no major sex differences was observed (57). The bone turnover markers peak in response to the pubertal growth spurt at the second to third Tanner stage, then the levels decrease in both boys and girls, suggesting a deceleration of skeletal growth and bone modeling and the substitution of bone remodeling as the main activity for bone metabolism (55, 59, 60). Overall, the levels of bone turnover markers are higher than that of adults throughout childhood.

1.5 Hormonal and metabolic effects

Growth hormone (GH) and Insulin-Like Growth Factor-I (IGF-I) are the main regulators of skeletal growth during infancy and childhood. GH produced in the anterior pituitary controls the secretion of IGF-I, while the negative feedback mechanism of IGF-I inhibits GH secretion directly and indirectly by stimulating the release of somatostatin (61, 62). GH stimulates the proliferation and differentiation of the osteoblastic and chondrocytic lineage cells, and consequently promoting the bone formation process. IGF-I as the main mediator of the GH action enhances the function of the osteoblasts at a later stage of maturation (63). The GH/ IGF-I axis is not only a main regulator in the longitudinal bone growth, skeletal maturation and bone mass acquisition in childhood, but also important for the maintenance of bone mass and architecture in adulthood (61). Childhood GH deficiency is associated with short stature and poor bone density (64).

The activation of the hypothalamus–pituitary–gonadal axis during puberty results in the production of sex steroids androgens and estrogens, which contribute to the bone mineralization and the sexual dimorphism of skeletal growth (61). The stimulatory effect of androgens for periosteal bone expansion is greater in boys compared to girls, whereas estrogens show the inhibitory effect for periosteal expansion. In girls, estrogens also widen the marrow cavity by attenuating remodeling activities at the endocortical surface, resulting in greater trabecular bone area (61, 65). At the beginning of puberty, relatively low levels of

estrogen and testosterone activate the GH/IGF-1 axis to stimulate longitudinal bone growth. High levels of sex steroids at the end of puberty decelerate and then cease longitudinal bone growth (66). Estrogen deficiency causes prolonged skeletal growth and low BMD, whereas excess estrogen leads to early puberty and epiphyseal closure resulting in short stature (61).

Parathyroid hormone (PTH) secreted by the parathyroid chief cells is stimulated by the decreased circulating calcium. It serves to increase calcium and decrease phosphorus concentrations in the blood by coordinating the actions of the skeleton, intestine and kidney (61, 67). At the level of bone tissue, it exhibits both anabolic and catabolic actions and increases bone turnover. PTH stimulates the production of 1,25-dihydroxyvitamin D ($1,25(OH)_2D$), while in turn $1,25(OH)_2D$ and its inactive form of 25-hydroxyvitamin D (25(OH)D) suppress the PTH levels in addition to circulating calcium. The circulating vitamin D level plays a critical role in the calcium and phosphate homeostasis that corresponds with the actions of PTH. Vitamin D target tissues e.g., bone cells and adipose cells express a $1,25(OH)_2D$ receptor (68). The main role of vitamin D in promoting bone mineralization is through increasing intestinal calcium and phosphate absorption, or directly affecting osteoblasts to increase bone formation. In vitamin D deficiency, the increase in PTH secretion is probably the main determinant for bone loss (69).

The endocrine role of adipose tissues in altering bone remodeling is gaining attention owing to its effects on the osteoblasts and osteoclasts (70). Aside from extra-gonadal estrogen synthesis, adipocytes also expressed adipokines e.g., adiponectin and leptin. In vivo and vitro studies, adiponectin has been described to increase bone formation and reduce bone resorption, suggesting a protective effect on bone. However, the effect of leptin appears to be complex and vary by skeletal site (71). In epidemiological studies, an inverse association of adiponectin and a positive association of leptin with BMD have been reported in healthy children (72, 73) and adults (74). Moreover, adipokines are involved in inflammation and glucose homeostasis. Elevated inflammatory factors e.g., interleukin-6, C-reactive protein and the reduced insulin levels have been related to low BMD in children, adolescents and adults (75, 76).

1.6 Modifiable determinants on bone

The beneficial impact of physical activity particularly weight-bearing exercises and high-impact activities on bone mass and density in children and adolescents have been well-described (6). A recent systematic review of forty observational studies in 6 to 18 year-old children indicates that accelerometer-based moderate to vigorous physical activity (MVPA) is positively associated with bone outcomes, particularly in males and during peri-pubertal period (77). However, evidence on the associations between physical activity and bone structure and strength is less clear, some studies using calcaneal QUS were mainly based on cross-sectional data. In general, positive associations of self-reported (78) and objectively measured physical activity (79) with SOS, BUA and stiffness index have been reported. A cross-sectional study and a case-control study nested in the IDEFICS cohort also emphasized the positive associations of accelerometer-based MVPA, reported leisure time physical activity and weight-bearing exercises in sports clubs with stiffness index among children aged 2 to 10 years (80, 81). However, some studies observed that physical activity was cross-sectionally associated with stiffness index only in boys (82), or longitudinally associated with SOS only in girls (53). Nevertheless, a nine-month school-based intervention in pre- and early-pubertal girls only found positive effects from jumping and martial art on BUA rather than stiffness index (83). Until now, there is a paucity of longitudinal studies using calcaneal QUS and a lack of data investigating how light physical activity influences QUS indices.

Evidence regarding the deleterious effect of sedentary behavior on bone is inconclusive. Several studies using DXA suggested a slight and inverse association between objectively measured sedentary time and lower extremity bone mass and density, whereas little association was observed with peripheral QCT bone parameters (84, 85). A longitudinal study used high-resolution peripheral QCT even observed protective effects of sedentary time on bone microstructure i.e., trabecular thickness, cortical porosity, thickness and vBMD at the tibia (86). In previous investigations exploiting cross-sectional data from the IDEFICS study, accelerometer-based sedentary time was negatively associated with stiffness index only in 6 to 10 year-old children rather than in 2 to 5 year-old children, and no association was observed

between screen time and stiffness index (80). Besides, sedentary time was associated with poor stiffness index in the case-control study (81). Another cross-sectional study in Japanese children aged 10 to 11 years reported a negative association between accelerometer-based sedentary time and stiffness index in girls but not in boys (87). Moreover, studies on extreme examples of sedentary behavior such as daytime napping and nocturnal sleep have been reported in young and older adults, suggesting that extreme sleep duration (short and long) (88), long daytime napping (89) and poor sleep quality (90) increased the risk of low BMD and osteoporosis. However, only few studies have been conducted in children and adolescents, reporting a positive (91) or no associations (92, 93) of sleep duration with BMC and aBMD.

Adequate dietary calcium intake is critical for optimal bone health throughout life. Dairy products e.g., milk, yogurt and cheeses are not only the primary source of dietary calcium in the western diet, but also rich in other nutrients e.g., protein, vitamin D and phosphorus that are essential for bone health (94). In children and adolescents, a small but positive effect of calcium supplementation has been reported in randomized controlled trials, suggesting 0.57% to 5.80 % increased BMC or aBMD through supplements, and 3.2% to 19.0% through calcium-fortified foods at several skeletal sites (6). However, the associations between habitual calcium intakes, dairy products consumption and bone health indicators are relatively insufficient in observational studies (95). In the studies with calcaneal ultrasound measurements, positive associations of total dairy consumption with SOS and stiffness index but not BUA were observed in Flemish children aged 6 to 12 years (79). Moreover, positive associations were observed for calcium intake with stiffness index in adolescent girls aged 14 to 18 years (96), and with BUA in school children aged 4 to 16 years (97). In Japanese children aged 10 to 15 years, annual increase of dairy products intake has been found to be positively associated with the increase of stiffness index (98). Nevertheless, neither serum calcium levels nor urine calcium/creatinine ratio were observed to be associated with poor stiffness index in the IDEFICS case-control study (81).

Vitamin D plays a critical role in dietary calcium absorption and calcium homeostasis (61, 68). Unlike other essential nutrients obtained from dietary sources, vitamin D derives from both sun exposure and dietary intake. Intestinally absorbed vitamin D from the diet is either

ergocalciferol (vitamin D_2) from plant sources or cholecalciferol (vitamin D_3) from animal sources. However, the majority of vitamin D_3 is synthesized from 7-dehydrocholesterol in the skin exposed to ultraviolet B irradiation. Vitamin D undergoes the first hydroxylation in the liver to produce 25(OH)D, which is the serum indicator of vitamin D status, and the second hydroxylation in the kidney to produce $1,25(OH)_2D$, which is the biologically active vitamin D metabolite. Therefore, vitamin D is not only a micronutrient but also a steroid hormone. Although the biological effects of vitamin D in promoting bone mineralization have been proven, the effectiveness of vitamin D supplementation in intervention studies among healthy pediatric populations is still inconclusive (6). Besides, optimal vitamin D status has been considered as the serum 25(OH)D at the level for maximal suppression of PTH secretion. However, the reported thresholds of 25(OH)D levels with beneficial effects on PTH, bone turnover markers, BMD and/or BMC range from 20 to 75 nmol/l in previous studies (99-101). In the IDEFICS case-control study, no statistically significant odds ratios for poor stiffness index were observed across the serum 25(OH)D tertiles of < 34.9 nmol/l and 34.9 to 50.7 nmol/l compared to >50.7nmol/l (81). As a consequence of the inconsistent evidence, there is still lack of consensus on vitamin D definitions (102).

Childhood obesity has been increasing dramatically worldwide, and the body mass index (BMI) and its sex- and age-specific reference values are widely used to screen for the overweight and obesity in children and adolescents (103). It is worth to note that aforementioned unfavorable behaviors of decreased physical activity (104), increased sedentary time (105), extreme sleep duration (106) and insufficient vitamin D (107) have also been considered as risk factors for childhood overweight/obesity; therefore, impaired bone health should be theoretically observed in children with overweight/obesity. On the contrary, previous studies reported increased bone size and density in overweight/obese children compared to thin/normal weight children (108, 109), implying a positive effect of weight status on bone health. These seemingly paradoxical associations are not surprising, due to the fact that the compensation of increased bone strength in overweight/obese children is not sufficient to support the overload from their body weight (110-112). However, weight status may influence the interpretation of the association between lifestyle-related modifiable factors and bone health that should be taken into consideration.

Another concern regarding weight status and bone health is that BMI cannot differentiate between fat mass and fat free mass. Evidence has been suggested that the higher bone mass, density and strength observed in children with BMI-defined overweight/obesity mainly attribute to the greater lean mass rather than to fat mass (113). However, the increased lean mass can be observed either in active children having more physical activity, or in inactive children having overweight/obesity, suggesting complexly combined and independent impacts of physical activity and lean mass on bone health. Furthermore, previous findings on the associations of fat mass with bone mass and strength were contradictory and vary across age, sex and maturity in children and adolescents (114). Similarly conflicting results have also been shown in studies using calcaneus QUS parameters. For example, Zulfarina et al. (78) found that fat mass was negatively associated with stiffness index in girls but not in boys. However, Heydenreich et al. (115) found that relative fat mass was positively related to stiffness index in both sexes of secondary school children. Forero-Bogotá et al. (116) reported that 9 to 17.9 year-old children with less fat mass were likely to have lower BUA. In the previous IDEFICS studies, Sioen et al. (117) observed a consistently inverse association between the body fat indicator of skinfold thickness and stiffness index in preschool children aged 2 to 5 years. Whereas a positive association was observed in primary school children aged 6 to 9 years, and this association was further reversed to inverse after adjusting for fat free mass.

1.7 Research significance and questions

Childhood and adolescence are the critical periods with both vulnerability and opportunity for skeletal linear growth and bone mineral acquisition. On one hand, high frequency of fractures was observed in children and adolescents, partly due to the linear skeletal growth outpacing the bone mineral accumulation, whereas the optimal bone mass and strength have been suggested to be protective factors for facture risk (118-120). On the other hand, optimizing bone accrual during growth is of great significance for the later life since the bone loss will occur during age after achieving peak bone mass (10). It has been suggested that if peak bone mass could be increased by one standard deviation, the risk of an osteoporotic facture may be reduced by 50% in post-menopausal women (121). Therefore, irrespective of unmodifiable

factors e.g., genetics and aging, understanding the effects of modifiable factors in children and adolescents will be very helpful for preventing current or future fractures and osteoporosis.

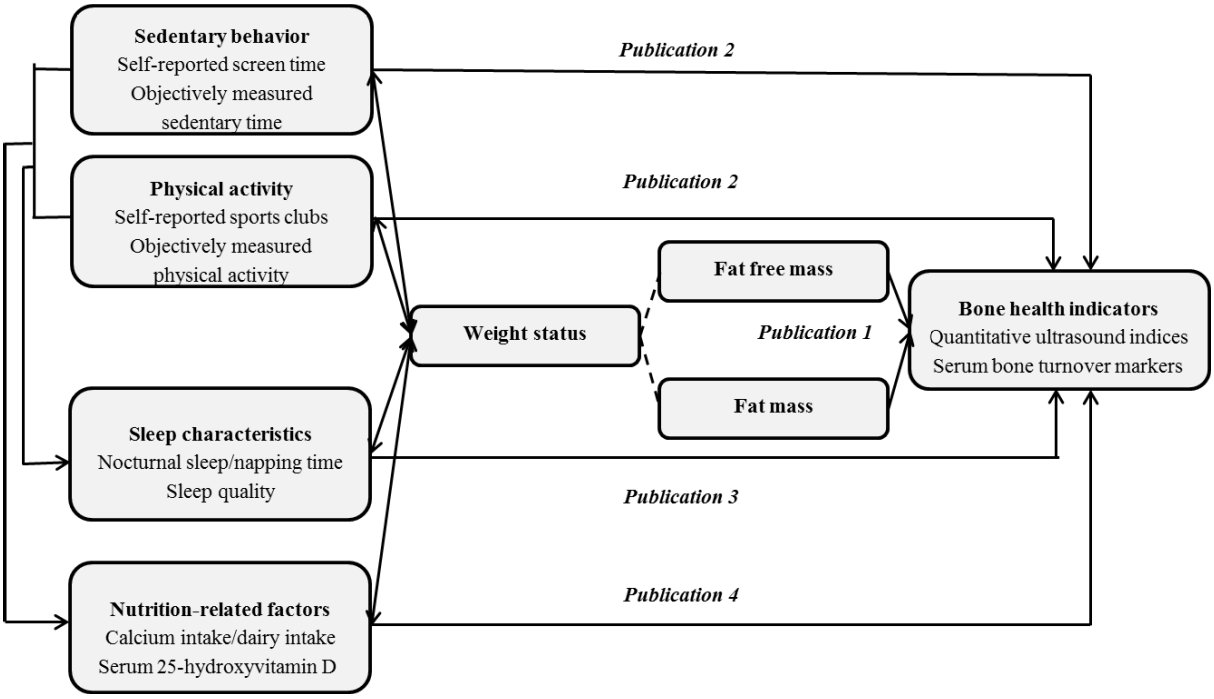
As the aforementioned findings from previous research, the associations of physical activity, sedentary behavior, sleep and nutrition with QUS indices and bone turnover in pediatrics remains unclear. The major research limitations and gaps result from: a) insufficient understanding on the longitudinal associations of habitual physical activity and different intensities of physical activity with stiffness index; b) uncertain effects of sedentary behavior in terms of total sedentary time and screen time on stiffness index; c) limited evidence on the associations between sleep duration, sleep quality and stiffness index; d) inconclusive findings on the relationships between vitamin D status, bone turnover markers and stiffness index; e) less studied modifying effect of weight status on the associations between behavioral risk factors and bone stiffness; f) controversial findings on the independent association between fat mass and bone stiffness while taking fat free mass into consideration.

2 Research hypotheses and aims

2.1 Conceptual framework

Based on existing research findings and the potential mechanisms underlying, a conceptual framework was developed to depict the hypotheses in the present thesis, and four publications were conceptualized in accordance with these hypotheses (Figure 1). I hypothesized that fat free mass would be positively associated with bone stiffness index, whereas this association might be attenuated by physical activity and sedentary behavior. In contrast, fat mass was hypothesized to be inversely associated with bone stiffness index (*Publication 1)*. Increased physical activity and reduced sedentary behavior were expected to be associated with higher bone stiffness index (*Publication 2*). Both short and long sleep duration was assumed to be associated with lower bone stiffness, and I hypothesized that these associations were independent of physical activity and sedentary behavior while may depend on sleep quality (*Publication 3*). Serum vitamin D level and calcium intake were hypothesized to be inversely associated with bone turnover markers while positively associated with bone stiffness index, and their associations were expected to be independent of physical activity and sedentary behavior (*Publication 4*). At last, BMI and its derived weight status were expected to be positively associated with bone stiffness index. Considering the bidirectional association between aforementioned behavioral factors and weight status, I hypothesized that their effect sizes on bone health indicators would differ by weight status; those were additionally explored by stratification and interaction in each publication manuscript.

Figure 1 Overview of the potential associations of sedentary behavior, physical activity, body composition, sleep and vitamin D with bone health indicators that are hypothesized and investigated in four publication manuscripts



2.2 Research aims and objectives

The present thesis aimed to investigate the complex relationships between modifiable factors and bone health in children and adolescents, in order to provide evidence for optimizing bone health in childhood and preventing osteoporosis fractures in the later life. To achieve these aims, I used sub-samples of children from the IDEFICS/I.Family cohort who provided at least one bone health indicator, either calcaneus stiffness index, or serum osteocalcin or CTx. The IDEFICS/I.Family study is a prospective cohort in European children and adolescents with a broad range of examinations including questionnaires, accelerometers, anthropometric measures, and biological samples etc. The specific objectives of each original paper were as follows:

- Investigating the longitudinal associations between body composition in terms of fat mass

and fat free mass and QUS-derived stiffness index, and their differences by sex and pubertal status (*Paper 1*).

- Investigating the cross-sectional and longitudinal associations of different dimensions of physical activity and sedentary behavior with QUS-derived stiffness index, and exploring the interplay of these associations with weight status (*Paper 2*).

- Investigating the cross-sectional and longitudinal associations between sleep duration, sleep quality and QUS-derived stiffness index, and exploring the interactions between nocturnal sleep duration, daytime napping and sleep quality (*Paper 3*).

- Investigating the cross-sectional associations of serum 25(OH)D with serum bone turnover markers osteocalcin and CTx and QUS-derived stiffness index, with consideration of physical activity, calcium intake and weight status (*Paper 4*).

3 Materials and methods

3.1 Study population

The IDEFICS/I.Family cohort is a multicenter population-based study conducted in eight European countries (Belgium, Cyprus, Estonia, Germany, Hungary, Italy, Spain and Sweden). The cohort provides repeated measurements of demographic characteristics, anthropometric parameters, biological markers, dietary and behavioral factors, aims to investigate the determinants of diet- and lifestyle-related diseases and disorders in children and adolescents. The first two examination waves were carried out in the context of the IDEFICS study: the baseline examination (wave 1) was conducted between September 2007 and May 2008 including 16,229 children aged 2 to 9.9 years; the second examination (wave 2) was conducted between September 2009 and May 2010 comprising 11,043 children from baseline and 2,543 newly recruited children (122). The third examination wave (wave 3) was carried out in the context of the I.Family study between January 2013 and June 2014 with a follow-up of 7,117 children from the original IDEFICS cohort and 2,501 newly recruited children (123). All the examinations were performed in accordance with the Declaration of Helsinki. Ethical approvals for all recruitment centers were obtained from their local ethics committees. All parents provided the signed informed consent before their children entered the study. In addition, children younger than 12 years gave their oral consent and children above 12 years provided a signed simplified form of consent prior to all exanimations.

In the IDEFCIS study, calcaneal QUS measurements of both feet were performed in eight participating countries including 7,539 children in wave 1 and 6,886 children in wave 2. In the I.Family study, QUS data were available for 2,892 children from five participating countries. I excluded children with invalid QUS measurements (i.e., the absolute difference of bone stiffness index between the left and right foot exceeds the 97th percentile of the study sample), and with indication of impaired bone health (i.e., medical conditions influenced the regular exercises and/or bone metabolism). The further inclusion and exclusion criteria for each paper varied in accordance with different research questions, exposures of interest, and

analysis strategies. The main sample sizes of the single papers in the present thesis are summarized in Table 1.

Table 1 Main sample sizes of the four papers in the present thesis

		Wave 1	Wave 2	Wave 3	N
Paper 1	Two-year longitudinal analysis [a]	✓	✓		2144
	Six-year longitudinal analysis [a]	✓		✓	833
Paper 2	Cross-sectional analysis [a]	✓			2008
	Two-year longitudinal analysis [a]	✓	✓		1653
	Six-year longitudinal analysis [a]	✓		✓	716
Paper 3	Cross-sectional analysis [a]		✓		4871
	Four-year longitudinal analysis [a]		✓	✓	861
Paper 4	Cross-sectional analysis [b]	✓			1171
	Cross-sectional analysis [c]	✓		✓	2481
	Cross-sectional analysis [a]	✓		✓	1123

Footnote: a. Bone stiffness index was used as the bone health outcome; b. Serum osteocalcin was used as the bone health outcome; c. Serum C-terminal telopeptides of type I collagen was used as the bone health outcome

3.2 Data collection

In the IDEFCIS/I.Family study, data from questionnaires and other examination modules were collected by trained nurses based on the standard operating procedures at the various examination waves. Questionnaires were answered by parents for their children younger than 12 years or self-reported by adolescents aged 12 years and older, including behavioral and socio-demographic information e.g., socioeconomic status, physical activity, sedentary behavior and diet. Anthropometric measurements related to weight status and body composition were performed, the standardization as well as intra- and inter-observer reliability of these measurements in the IDEFICS study has been published (124). Fasting venous blood was collected in the morning (between 8 a.m. and 10 a.m.) and then processed to separate serum and plasma. All the blood samples were under storage of -80 °C at a central bio-repository (125). Accelerometry to assess physical activity and ultrasonometry to assess calcaneal bone stiffness were only available in subsamples.

3.3 Bone health outcomes

3.3.1 Bone stiffness index

Bone stiffness index was measured on both right and left foot for each participant using the calcaneal QUS device (Lunar Achilles Insight^{TM} GE Healthcare, Milwaukee, WI). The Lunar Achilles OsteoReport Software was used to calculate stiffness index from the parameters SOS and BUA. Two different sizes of foot adapters were used to position the heels properly. Before recording each measurement, the preview image of the calcaneus was displayed on the screen with the default region of interest in order to avoid measurement error. In the previous reliability study, the reproducibility of two repeated measurements within a QUS device was assessed in a sub-sample of 60 children aged 5.6-9.3 years from the IDEFCIS baseline survey, suggesting the root-mean-square coefficient of variation for bone stiffness index on the left and right foot was 7.2% and 9.2%, respectively (126). For each participant, the mean value of stiffness index on the left and the right foot was calculated and further transformed to a sex-, age- and height-specific percentile based on the IDEFICS/I.Family reference population. The processing methods and reference values for the IDEFICS children have been published by Herrmann et al (57).

3.3.2 Bone turnover markers

The bone turnover markers considered in the *Paper 4* were serum CTx and osteocalcin. Serum concentrations of CTx (ng/ml) and total osteocalcin (ng/ml) were analyzed by chemiluminescence assays in the central laboratory on the Immunodiagnostic Systems iSYS (IDS-iSYS) automated analyser (Immunodiagnostic Systems GmbH, Frankfurt, Germany) using the IDS-iSYS CTX-I (CrossLaps®) and the N-MID® Osteocalcin assay, respectively. The unit of serum CTx was further converted to pg/ml (1 ng/ml = 1000 pg/ml) in order to have better interpretation of the regression coefficients.

3.4 Determinants

3.4.1 Weight status and body composition

Body height was measured to the nearest 0.1 cm using the standard clinical Seca 225 stadiometer (Seca, Hamburg, Germany); body weight was measured to the nearest 0.1 kg using the BC420 SMA scale (Tanita, Amsterdam, the Netherlands). BMI (kg/m^2) was calculated as weight divided by squared height. Age- and sex-specific z-scores of height, weight and BMI were calculated based on Cole et al. (127) Overweight and obesity was defined based on the extended International Obesity Task Force BMI criteria (128).

Skinfold thickness (mm) was measured twice at both triceps and subscapular site using Holtain Tanner/Whitehouse skinfold calipers (Holtain, Crosswell, UK; range 0–40 mm). The mean value of the two measurements at each site was calculated for later analyses. Fat mass (kg) was estimated based on Slaughter's equations by using subscapular and triceps skinfold thicknesses, which are preferred for assessment of body fat in children and youth (129, 130). Fat free mass (kg) was calculated as body weight minus fat mass. Age- and sex-specific z-scores of fat mass and fat free mass were derived based on the IDEFICS/I.Family reference population (131).

3.4.2 Physical activity and sedentary behavior

Subjectively measured physical activity was derived from the questions on the participation of sports clubs. If participants answered that the child was a member of a sports club, they had to report what kind of sport they did and how many hours and minutes per week they spent at the sports clubs. The category variable of weight-bearing exercises was defined according to the types of reported sports and classified into: (a) moderate or high mechanical loads on the lower limbs (ballgames, gymnastics, dancing, skating, martial arts, and athletics), and (b) no or low mechanical loads (swimming, biking and horseback riding) or no sports. The weekly time spent at sports clubs (hours/week) was calculated by adding reported hours and minutes. Subjectively measured sedentary behavior was derived from the questions on screen time. Participants reported the usual duration of the child watching TV/videos/ DVDs and playing

computer/game console on a normal weekday and weekend day. Six response categories were provided for questions and further converted as follows: not at all = 0, < 30 minutes = 0.25 hours, < 1 hour = 0.75 hours, 1- < 2 hours = 1.5 hours, 2-3 hours =2.5 hours, and > 3 hours = 4 hours. The weekly duration of watching TV/videos/ DVDs and playing computer/games (hours/week) was separately calculated by adding up the converted responses of weekdays and weekend days as follows: hours on weekdays/school days * 5 + hours on weekend days/vacations * 2. The weekly duration of screen time (hours/week) was the total duration of these two screen-based sedentary behaviors.

Objectively measured physical activity and sedentary time were measured using uniaxial accelerometers (GT1M or ActiTrainer, ActiGraph, Pensacola, FL, USA) in the IDEFCS study, and either a uniaxial (GT1M) or a triaxial accelerometer (GT3x+, ActiGraph, Pensacola, FL, USA) with the vertical axis outputs in the I.Family study. The sensor units of these models are identical. Participants were instructed to wear the accelerometer on the right hip with an adjustable elastic belt, and only to remove it during water-based activities and bedtime. All accelerometer data were re-integrated to a 60 seconds epoch. Valid measurements were defined as children who had at least 360 minutes of daily wearing time after exclusion of non-wear time for at least two valid weekdays and one valid weekend day. More details of accelerometer data processing in the IDEFICS/I.Family cohort can be found elsewhere (132, 133). Average minutes per day of time spent at various intensities were derived based on Evenson cut-off points as follows: sedentary time (≤100 cpm), light physical activity (101-2295 cpm), and moderate-to-vigorous physical activity (MVPA; ≥2296 cpm) (134). According to the WHO guidelines on Physical Activity and Sedentary Behavior for children and adolescents, average time spent in MVPA was classified into less than 60 minutes per day and at least 60 minutes per day.

3.4.3 Sleep characteristics

Information on nocturnal sleep and daytime napping in hours and minutes at weekdays/school days and weekend days/vacations was collected using a questionnaire. The average durations of nocturnal sleep and daytime napping was separately calculated for each child as follows:

(hours on weekdays/school days * 5 + hours on weekend days/vacations * 2) / 7. Total sleep duration was calculated as the sum of hours in nocturnal sleep and daytime napping. According to the sleep duration recommendation from the National Sleep Foundation (135), extreme sleep duration (short or long) for pre-school children aged 2 to < 6 years was defined as total sleep duration < 10 hours/day or ⩾ 13 hours/day; for primary school children aged 6 to < 12 years was < 9 hours/day or ≥ 11 hours/day; for adolescents aged 12 to15 years was < 8 hours/day or ≥ 10 hours/day.

Proxy indicators of sleep quality were derived from the questions on typical sleeping habits and daytime condition. Participants reported whether the child had a regular bedtime routine (yes = 1 and no = 0); had trouble getting up in the morning (yes = 0 and no = 1); and had difficulty falling asleep (yes = 0 and no = 1). The cumulative score was calculated by adding the numbers of these three items, and children who had three scores were considered as having good sleep quality.

3.4.4 Vitamin D and other dietary assessments

Serum 25(OH)D concentration (ng/ml) was used as an indicator of the vitamin D status, and analyzed by chemiluminescence assays in a central laboratory on the IDS-iSYS automated analyser (Immunodiagnostic Systems GmbH, Frankfurt, Germany) using the 25-Hydroxy Vitamin D^{s} assay.

Consumption frequency of dairy products was derived from a validated and reproducibility tested food frequency questionnaire by asking how many times the child consumed milk, yoghurt, cheese and butter in the last month (136-138). The responses categories for each food item were converted into weekly frequencies as follows: never/less than once a week = 0; 1-3 times per week = 2; 4-6 times per week = 5; once per week = 7; twice per day = 14; three times per day = 21; and four or more times per day = 30. The converted frequencies for consumption of all these dairy products were further summed up and expressed as frequencies per week.

Dietary calcium intake was estimated using the computer-based 24-hour dietary recalls those

were developed and validated in the IDEFICS and I.Family study (139, 140), called SACINA (Self-Administered Children and Infant Nutrition Assessment) and SACANA (Self-Administered Children, Adolescents, and Adult Nutrition Assessment), respectively. Briefly, participants reported the dietary intake in the previous 24 hours of the child by selecting country-specific food items and food combinations with standardized photographs of portion sizes. In the IDEFICS study, meals, drinks and snacks for children who had lunch at school were also recorded by trained personnel using the standardized observer sheet. To compute the nutrients intake, simple foods or European homogeneous multi-ingredient food items were linked to the German food composition table (German Nutrient Data Base, Version II.3.1). Usual intake of calcium (mg/day) was further estimated based on the U.S. National Cancer Institute Method (141, 142), with adjustments of age, sex, consumption frequency of dairy products and BMI to improve the estimates. Besides, repeated recalls that were available for a subgroup of children were used to correct the intra-individual daily variations. More details on dietary data process and usual intakes have been published by Börnhorst et al. (143) According to the population reference intake for calcium from the European Food Safety Authority, daily calcium intake of at least 450 mg for children aged 1 to 3 years, 800 mg for 4 to 10 years, and 1150 mg for 11 to 17 years was defined as sufficient (144).

3.4.5 Confounders

Sex, age, family socioeconomic status, sunlight exposure (daylight duration/ultraviolet radiation index) and pubertal status were considered as potential confounders in the present thesis. The highest educational level of parents was recorded as an indicator for family socioeconomic status according to the International Standard Classification of Education and categorized into low, medium and high (145). Sunlight exposure not only accounts for the main source of vitamin D synthesis, but also influences the circadian rhythm and outdoor plays etc.(146) Therefore, mean daylight duration (± 0.1 h) for each examination month in each location was calculated using astronomical tables as a proxy for sunlight exposure in the *Paper 1, 2 and 3*. However, ultraviolet radiation index of the month previous to the month of blood sampling was used as a proxy indicator in *Paper 4*, as vitamin D synthesis is mainly in

response to ultraviolet B radiation from sunlight. Self-reported voice change for boys and first menstrual period for girls was recorded to define pubertal status (147).

3.5 Statistical methods

All the statistical analyses were conducted with SAS software (version 9.3 or 9.4; SAS Institute, Inc., Cary, NC). In the descriptive statistics, continuous variables were presented as mean and standard deviations; and the differences between groups were compared with t-tests, associations between two continuous variables were estimated with Pearson's correlations. Categorical variables were presented as frequencies and percentages, and the differences between categories were compared with chi-square tests.

Linear mixed effects models (SAS procedure PROC MIXED) were used to evaluate the cross-sectional and longitudinal associations between exposures of interest and bone health outcomes with adjustment of potential confounders; a random effect for countries was added in all models to account for cluster effects.

For the cross-sectional analysis, data from wave 1 were used as baseline in *Paper 1* while in *Paper 3* data from wave 2 were used as baseline. In *Paper 4,* pooled data from wave 1 and wave 3 were analyzed with included a repeated statement in PROC MIXED, since some participants provided data in both waves.

For the longitudinal analysis, two-year (wave 2-wave 1) and six-year change (wave 3-wave 1) of the stiffness index percentiles was considered as the dependent variable in *Paper 1* and *Paper 2,* baseline and corresponding changes of exposures were included as independent variables. *In Paper 3*, the percentiles of stiffness index at four years follow-up (wave 3) was considered as the dependent variable; the exposures at baseline (wave 2) and follow-up (wave 3) were included as independent variables.

4 General results and discussion

4.1 Weight status, body composition and bone

Excess body weight can increase the bone mass and alter bone architecture in response to the increased mechanical loading (109, 148). In *Paper 4*, similar results in school-age children and adolescents were observed, suggesting that participants with overweight/obesity had higher stiffness index while lower serum CTx compared to those with thin/normal weight. However, overweight/obese children are also likely to have higher absolute muscle mass but lower muscle strength relative to body mass (149). Therefore, Slaughter's equations for skinfold thickness were applied in *Paper 1* to separate body weight into fat mass and fat free mass, the latter has been suggested to be a good proxy indicator for skeletal muscle mass and strength in healthy children (150). It is shown that baseline and the changes of fat free mass z-scores were strongly and positively associated with changes of stiffness index percentiles after two-year and six-year follow-up. On one hand, this result adds to existing evidence on bone mass highlighting the beneficial effect of body weight on bone stiffness is predominantly caused by fat free mass (113). On the other hand, it implies that QUS-derived stiffness index is a good indicator for bone mass and strength, due to the fact that the skeletal muscle and bone as the two largest tissues of the musculoskeletal system are closely related.

Compared to the strong and independent effect of fat free mass on stiffness index, the association between gain in fat mass and stiffness index varied according to sex and pubertal status. In *Paper 1*, an inverse association was observed over the six-year follow-up period between change of fat mass z-scores and stiffness index percentiles in boys and girls before menarche. Several possible mechanisms may explain the detrimental effect of fat on bone growth. First, high levels of adipose tissue have been linked to the reduction of GH/IGF-1 secretion even regardless of weight status, which may result in impaired bone growth (151). Moreover, insulin resistance as a common metabolic complication of adiposity has been suggested to mediate the inverse association of fat mass and BMC (152); it may suppress the IGF-1 production and consequently leads to impaired cortical bone strength (153). In contrast, the weak positive association between fat mass z-scores and bone stiffness percentiles in girls

after menarche is partly compatible to existing evidence in young and elderly women, suggesting that fat mass appears to be a protective factor for low BMD and osteoporosis (154). The discrepancy in females before and after menarche may be explained with the rapid rise of estrogens during puberty. In parallel to high levels of estrogens, increased androgens related to fat gain are also shown in pubertal girls. These alterations of sex steroid may lead to increased bone mineral accrual (155). Besides, increased leptin level in obese children has been indicated to positively associate with bone turnover and inversely associate with radial cortical porosity (156, 157). Leptin may also have an indirect effect on bone formation by activating estrogens (158). Nevertheless, the different effects and interplays of body fat and fat-induced cytokines on bone remain uncertain.

4.2 Interplay of weight status with sedentary behavior and bone stiffness

Sedentary lifestyles (i.e., total sedentary time, television viewing, computer use, and video game playing) have been identified as important risk factors for childhood obesity (159), which may in turn compensate the reduced mechanical loading. This leads to the hypothesis that the unfavorable effect of sedentary behavior on bone health may be underestimated because of overweight/obesity. The cross-sectional results from *Paper 2* supports this hypothesis, suggesting that weekly duration of watching TV/video/DVD was inversely associated with stiffness index in children having thin/normal weight at baseline, meanwhile sedentary time was inversely associated with stiffness index in children with overweight/obesity. Comparable results have been reported in a recent cross-sectional study among children aged 10 to 14 years, showing that the time spent using computer was negatively correlated to lumbar aBMD only in normal weight children (160). In addition, the longitudinal results of *Paper 2* showed that weekly duration of watching TV/video/DVD was inversely associated changes in stiffness index percentiles in children with overweight/obesity after two years, and weekly duration of playing computer/games was inversely associated with changes in stiffness index percentiles after six years. This finding for the first time revealed a shift of association between specific patterns of screen-based sedentary behavior and bone stiffness as children get older, which is meaningful for the development of more effective interventions to improve bone health.

As the deleterious effect of sedentary behavior on bone is mainly due to the reduced mechanical loading and sunlight exposure, it is still questionable whether the osteogenic effect of physical activity could compensate for the lack of mechanical stimuli or reduced vitamin D synthesis. Moreover, animal experiments showed that the interpolation of rest bouts during mechanical stimulus is also crucial to bone formation and strength (161, 162). An observational study in youth aged 8 to 22 years reported that non screen-based sedentary behavior might have beneficial effect on BMC if taking the intermittence between sedentary behavior and physical activity into consideration (163). Further studies should put more emphasis on the combined effects and the alternation of sedentary behavior and different physical activity on bone strength in children and adolescents.

4.3 Osteogenic effect of physical activity

Except for the positively cross-sectional associations between objectively measured MVPA and stiffness index which were in line with the previous IDEFICS studies (80, 164), the present thesis adds to the limited evidence on the longitudinal relationship between objectively measured MVPA and stiffness index. In *Paper 2,* baseline and change in MVPA were observed to be positively associated with two-year and six-year changes in stiffness index percentiles. Comparable but relatively weaker associations were observed on the cross-sectional and longitudinal association between time spent in sports clubs and stiffness index percentiles. In addition, my finding supports the beneficial effect of meeting the WHO recommendation with MVPA at least 1 hour per day on stiffness index percentiles. For those meeting the recommendation at both time points, higher increases of stiffness index percentiles with 10.39 units after two years and 12.68 units after six years were observed compared to their counterparts. Conversely, the effect of objectively measured light physical activity on stiffness index was negligible. However, few studies reported a positive association between light physical activity and whole-body aBMD and BMC (165, 166), while another longitudinal study reported an inverse association of light physical activity with aBMD and BMC at whole body, lumbar spine and femoral neck (167). Up to now, the impact of light physical activity on specific bone site and structural strength and the underlying mechanisms are not fully understood.

It has been established in animal models that the gain in bone mass and structural strength generated by physical activity is a response to the high-intensity loading forces (168). Some endocrine changes may also contribute to the skeletal adaptation of physical activity. For example, a longitudinal study suggested that the positive association of MVPA with BMC and aBMD may be explained by the decreased leptin level in pubertal boys (32). Moreover, physical activity has been linked to the increase of GH/IGF-I activation, presenting a more pronounced effect in advanced pubertal stages (169). However, the results in *Paper 2* was not enough to support the conclusion from the previous cross-sectional study and case-control study within the IDEFICS cohort, which reported that weight-bearing exercises substantially contributed to a higher stiffness index. The lack of statistical significance in the present thesis may be due to the considerably reduced sample sizes, since only children who had follow-up data were included in the analysis. Nevertheless, I observed a higher but not statistically significant increase of stiffness index percentiles in children had weight-bearing exercises over the six years of follow-up, and the effect size was observed to be more pronounced in overweight/obese group compared to the thin/normal weight group.

It is widely acknowledged that physical activity stimulates the bone formation. However, the changes in bone turnover related to physical activity are not fully understood in pediatrics. Few studies conducted in youth with several types of training program reported overall elevated bone turnover, or an increase of bone formation along with a decrease or no change of bone resorption (170, 171). In *Paper 4*, I fill the knowledge gap on the relationship between physical activity and bone turnover markers in healthy growing children, suggesting that meeting the MVPA recommendation was observed to be associated with increased osteocalcin in children aged 2 to 6 years; time spent in sports clubs was positively while total screen time was negatively associated with CTx in children aged 12 to 15 years. Higher bone turnover in these critical periods may contribute to the accelerated skeletal modeling, implying a window of opportunity for bone mineral accrual.

4.4 Sleep and bone stiffness

Sleep plays an important role in the health development of children and adolescents. Extreme

sleep duration including too short or too long in childhood has been linked to cardiovascular risks in the form of obesity (172), insulin resistance (173) and high blood pressure (174) etc. However, there remains a paucity of studies examining the relationship between sleep duration and bone health in children. In *Paper 3*, I observed that nocturnal sleep duration was positively associated with stiffness index only in children with adequate sleep duration. Meanwhile, daytime napping could partly make up for the unfavorable effect of short total sleep duration on stiffness index. Besides, baseline extreme sleep duration was observed to predict lower stiffness index after four-year follow-up in children with poor sleep quality. Even though the needs of sleep duration vary among individuals and change as children grow older, optimal sleep characteristics that having regular bedtime routine, no trouble falling asleep at night and getting up in the morning may be helpful for identifying adequate sleep (175, 176). My findings emphasize the importance of examining multiple sleep dimensions simultaneously to get a better understanding of sleep and bone in childhood.

Extreme sleep duration and poor quality always parallel with the interruption of circadian rhythm. Experimental studies have demonstrated the existence of clock genes in bone cells and the daily rhythm in bone turnover markers, suggesting that the disturbances of sleep and circadian could potentially disrupt bone physiology and thereby impair bone health (177). Moreover, cortisol levels were generally suppressed during sleep. The increased cortisol because of short sleep can exert detrimental effects either directly on the musculoskeletal system or via inhibiting GH and IGF-1 (178, 179). Besides, long sleep duration has been demonstrated to be associated with increased inflammatory factors in a recent systematic review (180).

Sleep time together with other physical behaviors i.e., sedentary time, light physical activity and MVPA contribute to the integrated 24-hour activity. In *Paper 4*, I was able to describe for the first time that the relationship between self-reported sleep duration and stiffness index was independent of reported time spent in sports clubs and screen time. However, few studies investigated the combined effects of 24-h movement behaviors on bone health using 24-h accelerometer data and compositional analyses. For example, a longitudinal study followed up children from 1 to 5 years of age, reported a positive association of the proportion of

MVPA at 2 and 3.5 years with BMC and aBMD at 5 years, while no associations were observed between other compositional time of 24-hour day and bone (93). Another cross-sectional study in children aged 10 to 12 years reported that the days with less sedentary time, moderately light physical activity as well as more sleep and MVPA were beneficial for pQCT parameters at tibia, and the estimated optimal sleep duration ranged from 9 to 11 hours (181). In the last five years, several 24-hour movement guidelines for infants, children and adolescents have been developed and released in Canada, Australia and WHO etc. (182), further studies are still needed to confirm the benefits of meeting these guidelines with regard to bone health.

4.5 Nutrition-related factors and bone health indicators

Maintaining adequate vitamin D and calcium intake is essential for stabilizing PTH levels and optimizing bone strength (6). Even though patients with vitamin D deficiency present an increase in bone turnover, the rickets caused by vitamin D deficiency or vitamin D receptor mutation can be counteracted by supplying adequate amounts of calcium and phosphate, demonstrating that the direct vitamin D effect on bone is relatively modest while the indirect effect is more pronounced (68). In *Paper 4*, vitamin D sufficiency at a cut-off of 20 ng/ml was found to be a protective factor for calcaneal stiffness index in children meeting the MVPA guideline for average 60 minutes per day. This finding points towards the possibility that the health effect of vitamin D on bone is in conjunction to MVPA levels. However, it is uncertain that the enhanced effect of vitamin D is because of the outdoor activities or MVPA in general. Evidence is also limited from either intervention studies of vitamin D supplementations or observational studies of vitamin D status in supporting skeletal health combined with physical activity in childhood (6, 183). Moreover, a stronger inverse association was observed between serum 25(OH)D and bone resorption marker CTx in contrast to bone formation marker osteocalcin. However, the actions of vitamin D in the modulation of bone turnover may be mainly mediated by PTH secretion (68), which has been reported to be inversely associated with 25(OH)D while positively associated with bone formation (184, 185). Further researches are needed to study the effects of vitamin D as well as the modulatory effect of PTH on bone turnover and mineral accrual.

The beneficial effect of milk and dairy products on bone health has been characterized by its inverse association with bone turnover markers and positive association with BMC (94). The results in *Paper 4* from the cross-sectional analyses in pooled data from wave 1 and wave 3 are congruent with previous findings, showing that dairy intake measured by food frequency questionnaire and usual calcium intake measured by 24-hour dietary recalls were negatively associated with serum CTx in all age groups, while positively associated with bone stiffness in children aged from 7 to 15 years. In addition, these associations were independent of physical activity, sedentary behavior and BMI. Nevertheless, more and more attention has been paid to the combined effect or interactive effect between milk or dairy intake and physical activity on BMC and aBMD in observational studies (166, 186). The combined effect on BMC and aBMD of exercise and calcium intake interventions in children also showed to be greater compared to either exercise or calcium intake alone (187). However, we still do not know whether vitamin D status modifies the bone response to calcium and PA.

4.6 Broadband ultrasound attenuation and speed of sound

I further conducted a sensitivity analysis using BUA and SOS as bone health outcomes based on the study samples and methods in *Paper 4*, and observed similar associations compared with stiffness index percentile as the outcome. However, a stronger positive association between BUA and weight z-scores, while a stronger inverse association between SOS and height z-scores was observed (Table 2). This disparity was in line with previous findings, indicating that bone size was inversely correlated with SOS values, and SOS may be related more strictly to bone density while BUA be related more strictly to bone structure (20, 188). Moreover, I found that SOS and BUA are highly correlated with stiffness index percentile, and they showed similar inverse correlations with CTx as stiffness index percentile did. These observations are comparable with previous findings in adults regarding the association between bone turnover markers and calcaneal QUS indices (189, 190).

Table 1 Cross-sectional associations of exposures with BUA, SOS and SI percentiles in 2013/2014 examination, stratified by age groups

	BUA		SOS		SI percentiles	
	6 to <12 years N=219	12 to 15 years N=276	6 to <12 years N=219	12 to 15 years N=276	6 to <12 years N=247	12 to 15 years N=299
Main sample [1]						
Serum 25(OH)D (ng/ml)	0.14(-0.11,0.40)	-0.02(-0.32,0.28)	0.33(-0.30,0.96)	0.50(-0.24,1.23)	0.52(-0.13,1.17)	0.36(-0.22,0.94)
Sports clubs (hours/week)	**0.65(0.07,1.23)**	**0.64(0.02,1.26)**	0.84(-0.56,2.25)	**2.02(0.56,3.47)**	1.21(-0.23,2.66)	**1.38(0.20,2.56)**
Screen time (hours/week)	-0.01(-0.19,0.16)	-0.10(-0.26,0.06)	-0.02(-0.45,0.42)	0.02(-0.36,0.40)	-0.20(-0.65,0.25)	-0.05(-0.36,0.25)
Dairy intake (frequency/week)	0.01(-0.08,0.10)	**0.11(0.02,0.21)**	0.03(-0.18,0.23)	**0.25(0.04,0.47)**	0.07(-0.15,0.29)	**0.20(0.04,0.36)**
Weight z-scores	**5.22(3.42,7.02)**	**6.68(4.91,8.45)**	3.58(-0.81,7.97)	3.99(-0.28,8.25)	**8.68(5.41,11.95)**	**5.77(2.83,8.71)**
Height z-scores	0.77(-0.89,2.42)	-1.52(-3.37,0.33)	-2.68(-6.72,1.35)	**-5.91(-10.38,-1.43)**	/	/
Sub-sample [2]	N=165	N=189	N=165	N=189	N=188	N=214
Serum 25(OH)D (ng/ml)	0.14(-0.18,0.45)	-0.02(-0.35,0.31)	0.34(-0.38,1.05)	0.42(-0.41,1.25)	0.36(-0.41,1.12)	0.24(-0.40,0.88)
MVPA (min/day)	0.05(-0.03,0.14)	0.08(-0.02,0.18)	0.12(-0.08,0.31)	0.23(-0.02,0.47)	**0.23(0.02,0.44)**	0.17(-0.01,0.36)
Sedentary time (min/day)	0.0002(-0.02,0.02)	-0.002(-0.002,0.02)	-0.02(-0.06,0.02)	-0.03(-0.08,0.02)	-0.01(-0.05,0.03)	-0.01(-0.05,0.03)
Usual calcium intake (mg/day)	**0.01(0.004,0.02)**	**0.01(0.004,0.02)**	-0.01(-0.03,0.01)	**0.03(0.002,0.05)**	0.01(-0.01,0.03)	**0.02(0.003,0.04)**
Weight z-scores	**3.77(1.61,5.93)**	**6.19(4.05,8.33)**	1.46(-3.45,6.38)	4.28(-1.04,9.61)	**6.54(2.47,10.61)**	**6.89(3.45,10.33)**
Height z-scores	0.88(-1.20,2.95)	-0.94(-3.05,1.17)	-0.72(-5.44,4.00)	-0.65(-6.00,4.69)	/	/

Footnote: Linear mixed-effects models (SAS procedure PROC MIXED) were used with sex, age, parental educational level, and ultraviolet radiation index included as confounders, a random effect for countries was taken into account in all models; 1. Main samples consist of children with full information on self-reported physical activity, sedentary behavior consumption frequency of dairy products and each quantitative ultrasound parameter; 2. Sub-samples consist of children had accelerometer data, calcium intake measured using 24-h dietary recall and each quantitative ultrasound parameter; *BUA* Broadband ultrasound attenuation, *SOS* Speed of sound, *SI* Stiffness index, *25(OH)D* 25-hydroxyvitamin D, *MVPA* Moderate-to-vigorous physical activity

4.7 Strengths and limitations

The major strength of the present thesis is the prospective study design with repeated measurements in the diverse European pediatric populations which exposed to a great range of different environments, lifestyles and diets. The wide age range of the participants from 2 to 15 years provided a unique opportunity to investigate associations passing through the critical and highly dynamic periods of bone growth and development e.g., puberty. For most of research questions longitudinal analysis could be conducted and the availability of repeated measurements with regard to both exposures of interest and QUS measurement in the same subjects allowed the assessment of temporal trends. Although the attrition was unavoidable in prospective cohort studies that decreased sample sizes over the two years and six years of follow-up, the comparably large sample size still allowed in-depth research on stratified analyses by sex, age groups and weight status etc.

Another important strength is the objective measurement of bone stiffness index for healthy children in a large-scale epidemiological study. Even though it is still not fully understood which specific bone properties were reflected by QUS-derived parameters, the strong and stable cross-sectional and longitudinal associations of fat free mass and physical activity with stiffness index support that calcaneal QUS is a reliable method to estimate bone health in children. In addition, serum bone metabolic markers were also available in a sub-sample of children to support the findings from QUS measurements, albeit only cross-sectional analysis was conducted. However, to my best knowledge, this is the first study in the healthy pediatric population investigating the association between nutritional factors and bone turnover markers in addition to QUS measurements with consideration of physical activity, sedentary behavior and weight status. In the future, more longitudinal and experimental studies are needed to provide evidence on casual inferences between these exposures and bone turnover markers, as well as their subsequent effect on bone stiffness.

A further strength is the use of comprehensive and harmonized data for physiological parameters, biochemical markers, dietary and physical behaviors as well as potential confounders. For instance, both subjectively and objectively measured physical activity and

sedentary behavior were available, allowing not only investigating the type, duration and intensity of these exposures, but also making comparison of associations among different methods with regard to bone health outcomes. However, it is worth to mention that accelerometer data was only available for a sub-sample and whose size diminished considerably at each follow-up; therefore the results may not be generalized for the whole pediatric population. Besides, information of sleep duration was derived from self-administrated questionnaire rather than actigraphy that most likely led to overestimation. Nevertheless, my investigation for the first time revealed the association between sleep duration and bone stiffness as well as its interplays with sleep habits. The results provided a research direction for further detailed investigation of sleep characteristics and underlying mechanism on bone health.

5 Conclusions

5.1 Public health implications

The present thesis demonstrates the beneficial short-term and long-term effects of physical activity and fat free mass on stiffness index. Moreover, having one hour per day of MVPA enhance the beneficial effect of adequate vitamin D on calcaneal stiffness index, suggesting that future intervention studies of vitamin D supplementation on bone should incorporate strategies to improve MVPA levels. School-age children and adolescents with overweight/obesity had higher stiffness index and lower bone resorption compared with their normal weight peers, but it was mainly due to the adaption of greater fat free mass but not fat mass. Conversely, fat mass gain was inversely associated with stiffness index gain in boys and pre-pubertal girls. Hence, maintaining optimal body composition and bone strength should be considered simultaneously in further childhood health promotion. In the meantime, the negative association between sedentary behaviors and bone stiffness may be underestimated due to the increased mechanical loading from being overweight/obesity, suggesting that weight status should be taken into account as an important confounding factor for the association of bone health with physical activity and sedentariness in epidemiological studies. In addition, my findings shed light on the limited evidence for the association between sleep and bone in childhood, suggesting that both sleep duration and sleep quality should be considered in order to improve stiffness index. Overall, the present thesis provides comprehensive evidence for future behavioral intervention combined with physical activity, sedentary behavior, sleep, vitamin D and calcium intake on bone, and the findings imply that the bone modeling may be more sensitive to the change of health behaviors at the age of 2 to 6 years and 12 to 15 years.

5.2 Future research directions

First, numerous studies have demonstrated the separate health effects of sleep, sedentary behavior and physical activity. However, the bone health implication of 24-h movement

behaviors is not well understood. More 24-h movement behavior data in children and adolescents are needed to examine both the combined and relative relationship between 24-h movement behaviors, bone metabolism and bone stiffness. Second, the association between body fat and bone health remains unclear, and the potential endocrine pathways induced by fat tissues show both positive and inverse associations with bone health indicators. It is still challenging to distinguish the different effects of total body fat, site-specific body fat and fat-induced cytokines on bone health during growth. Third, evidence from longitudinal studies on the association between vitamin D and bone health is still limited. In particular, the assessment of PTH should be considered in order to better interpret the associations and to identify the threshold for optimal vitamin D. Last but not least, comprehensive interventions and health promotion on bone health are needed to evaluate effectiveness and to identify the window of opportunities in the early life. Implementation strategies and dissemination approaches to improve the adherence of health behavioral guidelines and nutrition recommendations as well as to maintain a healthy body composition are also necessary in general pediatric populations.

References

1. Burr D, Allen M. Bone morphology and organization. Basic and applied bone biology. London (UK): Elsevier; 2003.
2. Clarke B. Normal bone anatomy and physiology. Clinical journal of the American Society of Nephrology. 2008;3(Supplement 3):S131-S9.
3. Marieb EN, Hoehn K. Bones and Skeletal Tissues. Human anatomy & physiology: Pearson education; 2007.
4. Bellido T, Plotkin LI, Bruzzaniti A. Bone cells. Basic and applied bone biology: Elsevier; 2019. p. 37-55.
5. Florencio-Silva R, Sasso GRdS, Sasso-Cerri E, Simões MJ, Cerri PS. Biology of bone tissue: structure, function, and factors that influence bone cells. BioMed research international. 2015;2015.
6. Weaver CM, Gordon CM, Janz KF, Kalkwarf HJ, Lappe JM, Lewis R, et al. The National Osteoporosis Foundation's position statement on peak bone mass development and lifestyle factors: a systematic review and implementation recommendations. Osteoporos Int. 2016;27(4):1281-386.
7. Allen MR, Burr DB. Bone Growth, Modeling, and Remodeling. Basic and Applied Bone Biology: Elsevier; 2019. p. 85-100.
8. Setiawati R, Rahardjo P. Bone development and growth. Osteogenesis and bone regeneration. 2019;10.
9. Seeman E. Bone modeling and remodeling. Critical reviews in eukaryotic gene expression. 2009;19(3):219-33.
10. Heaney RP, Abrams S, Dawson-Hughes B, Looker A, Marcus R, Matkovic V, et al. Peak bone mass. Osteoporos Int. 2000;11(12):985-1009.
11. Bouxsein ML. Bone quality: where do we go from here? Osteoporosis international. 2003;14(5):118-27.
12. Stagi S, Cavalli L, Iurato C, Seminara S, Brandi ML, de Martino M. Bone health in children and adolescents: the available imaging techniques. Clinical cases in mineral and bone metabolism. 2013;10(3):166.
13. Stagi S, Cavalli L, Iurato C, Seminara S, Brandi ML, de Martino M. Bone metabolism in children and adolescents: main characteristics of the determinants of peak bone mass. Clinical cases in mineral and bone metabolism : the official journal of the Italian Society of Osteoporosis, Mineral Metabolism, and Skeletal Diseases. 2013;10(3):172-9.
14. Ward KA, Link TM, Adams JE. Tools for measuring bone in children and adolescents. Bone Health Assessment in Pediatrics: Springer; 2016. p. 23-52.
15. Weber DR, Boyce A, Gordon C, Högler W, Kecskemethy HH, Misra M, et al. The Utility of DXA Assessment at the Forearm, Proximal Femur, and Lateral Distal Femur, and Vertebral Fracture Assessment in the Pediatric Population: 2019 ISCD Official Position. J Clin Densitom. 2019;22(4):567-89.
16. Leonard MB, Shults J, Elliott DM, Stallings VA, Zemel BS. Interpretation of whole body dual energy X-ray absorptiometry measures in children: comparison with peripheral quantitative computed tomography. Bone. 2004;34(6):1044-52.
17. Ammann P, Rizzoli R. Bone strength and its determinants. Osteoporosis international.

2003;14(3):13-8.
18. Adams JE, Engelke K, Zemel BS, Ward KA. Quantitative computer tomography in children and adolescents: the 2013 ISCD Pediatric Official Positions. Journal of Clinical Densitometry. 2014;17(2):258-74.
19. Whittier DE, Boyd SK, Burghardt AJ, Paccou J, Ghasem-Zadeh A, Chapurlat R, et al. Guidelines for the assessment of bone density and microarchitecture in vivo using high-resolution peripheral quantitative computed tomography. Osteoporos Int. 2020;31(9):1607-27.
20. Baroncelli GI. Quantitative ultrasound methods to assess bone mineral status in children: technical characteristics, performance, and clinical application. Pediatr Res. 2008;63(3):220-8.
21. Chin KY, Ima-Nirwana S. Calcaneal quantitative ultrasound as a determinant of bone health status: what properties of bone does it reflect? International journal of medical sciences. 2013;10(12):1778-83.
22. Krieg MA, Barkmann R, Gonnelli S, Stewart A, Bauer DC, Del Rio Barquero L, et al. Quantitative ultrasound in the management of osteoporosis: the 2007 ISCD Official Positions. J Clin Densitom. 2008;11(1):163-87.
23. Jaworski M, Lebiedowski M, Lorenc RS, Trempe J. Ultrasound bone measurement in pediatric subjects. Calcif Tissue Int. 1995;56(5):368-71.
24. Sundberg M, Gärdsell P, Johnell O, Ornstein E, Sernbo I. Comparison of quantitative ultrasound measurements in calcaneus with DXA and SXA at other skeletal sites: a population-based study on 280 children aged 11-16 years. Osteoporos Int. 1998;8(5):410-7.
25. Torres-Costoso A, Vlachopoulos D, Ubago-Guisado E, Ferri-Morales A, Cavero-Redondo I, Martínez-Vizcaino V, et al. Agreement Between Dual-Energy X-Ray Absorptiometry and Quantitative Ultrasound to Evaluate Bone Health in Adolescents: The PRO-BONE Study. Pediatr Exerc Sci. 2018;30(4):466-73.
26. Xu Y, Guo B, Gong J, Xu H, Bai Z. The correlation between calcaneus stiffness index calculated by QUS and total body BMD assessed by DXA in Chinese children and adolescents. Journal of bone and mineral metabolism. 2014;32(2):159-66.
27. Lucas R, Martins A, Monjardino T, Caetano-Lopes J, Fonseca J, Preedy V. Bone Markers Throughout Sexual Development: Epidemiological Significance and Population-Based Findings. Biomarkers in Bone Disease Dordrecht: Springer Netherlands. 2016:1-34.
28. Shetty S, Kapoor N, Bondu JD, Thomas N, Paul TV. Bone turnover markers: Emerging tool in the management of osteoporosis. Indian journal of endocrinology and metabolism. 2016;20(6):846.
29. Szulc P, Seeman E, Delmas PD. Biochemical measurements of bone turnover in children and adolescents. Osteoporos Int. 2000;11(4):281-94.
30. Fortes CM, Goldberg TB, Kurokawa CS, Silva CC, Moretto MR, Biason TP, et al. Relationship between chronological and bone ages and pubertal stage of breasts with bone biomarkers and bone mineral density in adolescents. Jornal de pediatria. 2014;90(6):624-31.
31. Orito S, Kuroda T, Onoe Y, Sato Y, Ohta H. Age-related distribution of bone and skeletal parameters in 1,322 Japanese young women. Journal of bone and mineral metabolism. 2009;27(6):698-704.
32. Vaitkeviciute D, Lätt E, Mäestu J, Jürimäe T, Saar M, Purge P, et al. Longitudinal

associations between bone and adipose tissue biochemical markers with bone mineralization in boys during puberty. BMC Pediatr. 2016;16:102.
33. Dalskov S, Ritz C, Larnkjær A, Damsgaard CT, Petersen RA, Sørensen LB, et al. Associations between adiposity, hormones, and gains in height, whole-body height-adjusted bone size, and size-adjusted bone mineral content in 8- to 11-year-old children. Osteoporos Int. 2016;27(4):1619-29.
34. Kouda K, Ohara K, Nakamura H, Fujita Y, Iki M. Predicting bone mineral acquisition during puberty: data from a 3-year follow-up study in Hamamatsu, Japan. Journal of bone and mineral metabolism. 2017;35(2):185-91.
35. Zürcher SJ, Borter N, Kränzlin M, Neyer P, Meyer U, Rizzoli R, et al. Relationship between bone mineral content and bone turnover markers, sex hormones and calciotropic hormones in pre- and early pubertal children. Osteoporos Int. 2020;31(2):335-49.
36. Simm PJ, Johannesen J, Briody J, McQuade M, Hsu B, Bridge C, et al. Zoledronic acid improves bone mineral density, reduces bone turnover and improves skeletal architecture over 2 years of treatment in children with secondary osteoporosis. Bone. 2011;49(5):939-43.
37. Mora S, Pitukcheewanont P, Kaufman FR, Nelson JC, Gilsanz V. Biochemical markers of bone turnover and the volume and the density of bone in children at different stages of sexual development. Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research. 1999;14(10):1664-71.
38. McCormack SE, Chesi A, Mitchell JA, Roy SM, Cousminer DL, Kalkwarf HJ, et al. Relative Skeletal Maturation and Population Ancestry in Nonobese Children and Adolescents. Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research. 2017;32(1):115-24.
39. McCormack SE, Cousminer DL, Chesi A, Mitchell JA, Roy SM, Kalkwarf HJ, et al. Association Between Linear Growth and Bone Accrual in a Diverse Cohort of Children and Adolescents. JAMA Pediatr. 2017;171(9):e171769.
40. Brown LB, Streeten EA, Shuldiner AR, Almasy LA, Peyser PA, Mitchell BD. Assessment of sex-specific genetic and environmental effects on bone mineral density. Genetic epidemiology. 2004;27(2):153-61.
41. Guéguen R, Jouanny P, Guillemin F, Kuntz C, Pourel J, Siest G. Segregation analysis and variance components analysis of bone mineral density in healthy families. Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research. 1995;10(12):2017-22.
42. Weaver CM, Peacock M. Skeletal Changes Across the Life Span. Basic and Applied Bone Biology: Elsevier; 2019. p. 189-202.
43. Kalkwarf HJ, Gilsanz V, Lappe JM, Oberfield S, Shepherd JA, Hangartner TN, et al. Tracking of bone mass and density during childhood and adolescence. J Clin Endocrinol Metab. 2010;95(4):1690-8.
44. Wren TA, Kalkwarf HJ, Zemel BS, Lappe JM, Oberfield S, Shepherd JA, et al. Longitudinal tracking of dual-energy X-ray absorptiometry bone measures over 6 years in children and adolescents: persistence of low bone mass to maturity. The Journal of pediatrics. 2014;164(6):1280-5.e2.
45. Riggs BL, Melton LJ, Robb RA, Camp JJ, Atkinson EJ, McDaniel L, et al. A population-based assessment of rates of bone loss at multiple skeletal sites: evidence for

substantial trabecular bone loss in young adult women and men. Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research. 2008;23(2):205-14.
46. Bailey DA, McKay HA, Mirwald RL, Crocker PR, Faulkner RA. A six-year longitudinal study of the relationship of physical activity to bone mineral accrual in growing children: the university of Saskatchewan bone mineral accrual study. Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research. 1999;14(10):1672-9.
47. Baxter-Jones AD, Faulkner RA, Forwood MR, Mirwald RL, Bailey DA. Bone mineral accrual from 8 to 30 years of age: an estimation of peak bone mass. Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research. 2011;26(8):1729-39.
48. Berger C, Goltzman D, Langsetmo L, Joseph L, Jackson S, Kreiger N, et al. Peak bone mass from longitudinal data: implications for the prevalence, pathophysiology, and diagnosis of osteoporosis. Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research. 2010;25(9):1948-57.
49. Gilsanz V, Perez FJ, Campbell PP, Dorey FJ, Lee DC, Wren TA. Quantitative CT reference values for vertebral trabecular bone density in children and young adults. Radiology. 2009;250(1):222-7.
50. Kirmani S, Christen D, van Lenthe GH, Fischer PR, Bouxsein ML, McCready LK, et al. Bone structure at the distal radius during adolescent growth. Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research. 2009;24(6):1033-42.
51. Nishiyama KK, Macdonald HM, Moore SA, Fung T, Boyd SK, McKay HA. Cortical porosity is higher in boys compared with girls at the distal radius and distal tibia during pubertal growth: an HR-pQCT study. Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research. 2012;27(2):273-82.
52. Gabel L, Macdonald HM, McKay HA. Sex Differences and Growth-Related Adaptations in Bone Microarchitecture, Geometry, Density, and Strength From Childhood to Early Adulthood: A Mixed Longitudinal HR-pQCT Study. Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research. 2017;32(2):250-63.
53. Lee M, Nahhas RW, Choh AC, Demerath EW, Duren DL, Chumlea WC, et al. Longitudinal changes in calcaneal quantitative ultrasound measures during childhood. Osteoporos Int. 2011;22(8):2295-305.
54. Szmodis M, Zsákai A, Bosnyák E, Protzner A, Trájer E, Farkas A, et al. Reference data for ultrasound bone characteristics in Hungarian children aged 7-19 years. Annals of human biology. 2017;44(8):704-14.
55. Ramírez-Vélez R, Ojeda-Pardo ML, Correa-Bautista JE, González-Ruíz K, Navarro-Pérez CF, González-Jiménez E, et al. Normative data for calcaneal broadband ultrasound attenuation among children and adolescents from Colombia: the FUPRECOL Study. Arch Osteoporos. 2016;11:2.
56. Zhu ZQ, Liu W, Xu CL, Han SM, Zu SY, Zhu GJ. Ultrasound bone densitometry of the calcaneus in healthy Chinese children and adolescents. Osteoporos Int. 2007;18(4):533-41.

57. Herrmann D, Intemann T, Lauria F, Mårild S, Molnár D, Moreno LA, et al. Reference values of bone stiffness index and C-terminal telopeptide in healthy European children. Int J Obes (Lond). 2014;38 Suppl 2:S76-85.
58. Jürimäe J. Interpretation and application of bone turnover markers in children and adolescents. Current opinion in pediatrics. 2010;22(4):494-500.
59. Bayer M. Reference values of osteocalcin and procollagen type I N-propeptide plasma levels in a healthy Central European population aged 0-18 years. Osteoporos Int. 2014;25(2):729-36.
60. Diemar SS, Lylloff L, Rønne MS, Møllehave LT, Heidemann M, Thuesen BH, et al. Reference intervals in Danish children and adolescents for bone turnover markers carboxy-terminal cross-linked telopeptide of type I collagen (β-CTX), pro-collagen type I N-terminal propeptide (PINP), osteocalcin (OC) and bone-specific alkaline phosphatase (bone ALP). Bone. 2021;146:115879.
61. Bellido T, Gallant KMH. Hormonal effects on bone cells. Basic and Applied Bone Biology: Elsevier; 2014. p. 299-314.
62. Giustina A, Mazziotti G, Canalis E. Growth hormone, insulin-like growth factors, and the skeleton. Endocr Rev. 2008;29(5):535-59.
63. Esposito S, Leonardi A, Lanciotti L, Cofini M, Muzi G, Penta L. Vitamin D and growth hormone in children: a review of the current scientific knowledge. Journal of translational medicine. 2019;17(1):87.
64. Högler W, Shaw N. Childhood growth hormone deficiency, bone density, structures and fractures: scrutinizing the evidence. Clinical endocrinology. 2010;72(3):281-9.
65. Nakamura T, Imai Y, Matsumoto T, Sato S, Takeuchi K, Igarashi K, et al. Estrogen prevents bone loss via estrogen receptor alpha and induction of Fas ligand in osteoclasts. Cell. 2007;130(5):811-23.
66. Vanderschueren D, Vandenput L, Boonen S, Lindberg MK, Bouillon R, Ohlsson C. Androgens and bone. Endocr Rev. 2004;25(3):389-425.
67. Gaffney-Stomberg E, MacArthur MR, McClung JP. Parathyroid Hormone (PTH) and the Relationship Between PTH and Bone Health: Structure, Physiology, Actions, and Ethnicity. In: Patel VB, Preedy VR, editors. Biomarkers in Bone Disease. Dordrecht: Springer Netherlands; 2017. p. 443-61.
68. Carmina E. Vitamin D: Biological Significance and Diagnosis of Mild Deficiency. In: Preedy VR, editor. Biomarkers in Bone Disease. Dordrecht: Springer Netherlands; 2016. p. 1-13.
69. Laird E, Ward M, McSorley E, Strain JJ, Wallace J. Vitamin D and bone health: potential mechanisms. Nutrients. 2010;2(7):693-724.
70. Cao JJ. Effects of obesity on bone metabolism. Journal of orthopaedic surgery and research. 2011;6(1):1-7.
71. Neumann E, Junker S, Schett G, Frommer K, Müller-Ladner U. Adipokines in bone disease. Nature reviews Rheumatology. 2016;12(5):296-302.
72. Garnett SP, Högler W, Blades B, Baur LA, Peat J, Lee J, et al. Relation between hormones and body composition, including bone, in prepubertal children. Am J Clin Nutr. 2004;80(4):966-72.
73. Sayers A, Timpson NJ, Sattar N, Deanfield J, Hingorani AD, Davey-Smith G, et al.

Adiponectin and its association with bone mass accrual in childhood. Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research. 2010;25(10):2212-20.
74. Biver E, Salliot C, Combescure C, Gossec L, Hardouin P, Legroux-Gerot I, et al. Influence of adipokines and ghrelin on bone mineral density and fracture risk: a systematic review and meta-analysis. J Clin Endocrinol Metab. 2011;96(9):2703-13.
75. Fantuzzi G. Adipose tissue, adipokines, and inflammation. Journal of Allergy and clinical immunology. 2005;115(5):911-9.
76. Giudici KV, Fisberg RM, Marchioni DML, Peters BSE, Martini LA. Crosstalk between bone and fat tissue: associations between vitamin D, osteocalcin, adipokines, and markers of glucose metabolism among adolescents. Journal of the American College of Nutrition. 2017;36(4):273-80.
77. Bland VL, Heatherington-Rauth M, Howe C, Going SB, Bea JW. Association of objectively measured physical activity and bone health in children and adolescents: a systematic review and narrative synthesis. Osteoporos Int. 2020;31(10):1865-94.
78. Zulfarina MS, Sharif R, Syarifah-Noratiqah SB, Sharkawi AM, Aqilah-Sm ZS, Mokhtar SA, et al. Modifiable factors associated with bone health in Malaysian adolescents utilising calcaneus quantitative ultrasound. PloS one. 2018;13(8):e0202321.
79. De Smet S, Michels N, Polfliet C, D'Haese S, Roggen I, De Henauw S, et al. The influence of dairy consumption and physical activity on ultrasound bone measurements in Flemish children. Journal of bone and mineral metabolism. 2015;33(2):192-200.
80. Herrmann D, Buck C, Sioen I, Kouride Y, Marild S, Molnar D, et al. Impact of physical activity, sedentary behaviour and muscle strength on bone stiffness in 2-10-year-old children-cross-sectional results from the IDEFICS study. Int J Behav Nutr Phys Act. 2015;12:112.
81. Herrmann D, Pohlabeln H, Gianfagna F, Konstabel K, Lissner L, Marild S, et al. Association between bone stiffness and nutritional biomarkers combined with weight-bearing exercise, physical activity, and sedentary time in preadolescent children. A case-control study. Bone. 2015;78:142-9.
82. Szmodis M, Bosnyák E, Protzner A, Szőts G, Trájer E, Tóth M. Relationship between physical activity, dietary intake and bone parameters in 10-12 years old Hungarian boys and girls. Cent Eur J Public Health. 2019;27(1):10-6.
83. Nogueira RC, Weeks BK, Beck BR. An in-school exercise intervention to enhance bone and reduce fat in girls: the CAPO Kids trial. Bone. 2014;68:92-9.
84. Koedijk JB, van Rijswijk J, Oranje WA, van den Bergh JP, Bours SP, Savelberg HH, et al. Sedentary behaviour and bone health in children, adolescents and young adults: a systematic review. Osteoporos Int. 2017;28(9):2507-19.
85. Osborn W, Simm P, Olds T, Lycett K, Mensah FK, Muller J, et al. Bone health, activity and sedentariness at age 11-12 years: Cross-sectional Australian population-derived study. Bone. 2018;112:153-60.
86. Gabel L, Macdonald HM, Nettlefold L, McKay HA. Physical Activity, Sedentary Time, and Bone Strength From Childhood to Early Adulthood: A Mixed Longitudinal HR-pQCT study. Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research. 2017;32(7):1525-36.

87. Yamakita M, Ando D, Akiyama Y, Sato M, Suzuki K, Yamagata Z. Association of objectively measured physical activity and sedentary behavior with bone stiffness in peripubertal children. Journal of bone and mineral metabolism. 2019;37(6):1095-103.
88. Wang D, Ruan W, Peng Y, Li W. Sleep duration and the risk of osteoporosis among middle-aged and elderly adults: a dose-response meta-analysis. Osteoporos Int. 2018;29(8):1689-95.
89. Saetung S, Reutrakul S, Chailurkit LO, Rajatanavin R, Ongphiphadhanakul B, Nimitphong H. The Association between Daytime Napping Characteristics and Bone Mineral Density in Elderly Thai Women without Osteoporosis. Sci Rep. 2018;8(1):10016.
90. Lucassen EA, de Mutsert R, le Cessie S, Appelman-Dijkstra NM, Rosendaal FR, van Heemst D, et al. Poor sleep quality and later sleep timing are risk factors for osteopenia and sarcopenia in middle-aged men and women: The NEO study. PloS one. 2017;12(5):e0176685.
91. Casazza K, Hanks LJ, Fernandez JR. Shorter sleep may be a risk factor for impaired bone mass accrual in childhood. J Clin Densitom. 2011;14(4):453-7.
92. Nakagi Y, Ito T, Hirooka K, Sugioka Y, Endo H, Saijo Y, et al. Association between lifestyle habits and bone mineral density in Japanese juveniles. Environ Health Prev Med. 2010;15(4):222-8.
93. Taylor RW, Haszard JJ, Meredith-Jones KA, Galland BC, Heath AM, Lawrence J, et al. 24-h movement behaviors from infancy to preschool: cross-sectional and longitudinal relationships with body composition and bone health. Int J Behav Nutr Phys Act. 2018;15(1):118.
94. Rizzoli R. Dairy products, yogurts, and bone health. Am J Clin Nutr. 2014;99(5 Suppl):1256s-62s.
95. Wallace TC, Bailey RL, Lappe J, O'Brien KO, Wang DD, Sahni S, et al. Dairy intake and bone health across the lifespan: a systematic review and expert narrative. Critical reviews in food science and nutrition. 2020:1-47.
96. Robinson ML, Winters-Stone K, Gabel K, Dolny D. Modifiable lifestyle factors affecting bone health using calcaneus quantitative ultrasound in adolescent girls. Osteoporos Int. 2007;18(8):1101-7.
97. Lavado-Garcia JM, Calderon-Garcia JF, Moran JM, Canal-Macias ML, Rodriguez-Dominguez T, Pedrera-Zamorano JD. Bone mass of Spanish school children: impact of anthropometric, dietary and body composition factors. Journal of bone and mineral metabolism. 2012;30(2):193-201.
98. Hirota T, Kusu T, Hirota K. Improvement of nutrition stimulates bone mineral gain in Japanese school children and adolescents. Osteoporos Int. 2005;16(9):1057-64.
99. Hill TR, Cotter AA, Mitchell S, Boreham CA, Dubitzky W, Murray L, et al. Vitamin D status and parathyroid hormone relationship in adolescents and its association with bone health parameters: analysis of the Northern Ireland Young Heart's Project. Osteoporos Int. 2010;21(4):695-700.
100. Outila TA, Kärkkäinen MU, Lamberg-Allardt CJ. Vitamin D status affects serum parathyroid hormone concentrations during winter in female adolescents: associations with forearm bone mineral density. Am J Clin Nutr. 2001;74(2):206-10.
101. Wu F, Laslett LL, Zhang Q. Threshold Effects of Vitamin D Status on Bone Health in Chinese Adolescents With Low Calcium Intake. J Clin Endocrinol Metab.

2015;100(12):4481-9.
102. Moon RJ, Harvey NC, Davies JH, Cooper C. Vitamin D and skeletal health in infancy and childhood. Osteoporos Int. 2014;25(12):2673-84.
103. Güngör NK. Overweight and obesity in children and adolescents. J Clin Res Pediatr Endocrinol. 2014;6(3):129-43.
104. Hills AP, Andersen LB, Byrne NM. Physical activity and obesity in children. British journal of sports medicine. 2011;45(11):866-70.
105. Rey-López JP, Vicente-Rodríguez G, Biosca M, Moreno LA. Sedentary behaviour and obesity development in children and adolescents. Nutrition, metabolism and cardiovascular diseases. 2008;18(3):242-51.
106. Felső R, Lohner S, Hollódy K, Erhardt É, Molnár D. Relationship between sleep duration and childhood obesity: Systematic review including the potential underlying mechanisms. Nutrition, Metabolism and Cardiovascular Diseases. 2017;27(9):751-61.
107. Pereira-Santos M, Costa PRdF, Assis AMOd, Santos CAdST, Santos DBd. Obesity and vitamin D deficiency: a systematic review and meta-analysis. Obesity reviews. 2015;16(4):341-9.
108. Fintini D, Cianfarani S, Cofini M, Andreoletti A, Ubertini GM, Cappa M, et al. The Bones of Children With Obesity. Front Endocrinol (Lausanne). 2020;11:200.
109. van Leeuwen J, Koes BW, Paulis WD, van Middelkoop M. Differences in bone mineral density between normal-weight children and children with overweight and obesity: a systematic review and meta-analysis. Obes Rev. 2017;18(5):526-46.
110. Goulding A, Taylor RW, Jones IE, McAuley KA, Manning PJ, Williams SM. Overweight and obese children have low bone mass and area for their weight. Int J Obes Relat Metab Disord. 2000;24(5):627-32.
111. Mosca LN, da Silva VN, Goldberg TB. Does excess weight interfere with bone mass accumulation during adolescence? Nutrients. 2013;5(6):2047-61.
112. Rocher E, Chappard C, Jaffre C, Benhamou CL, Courteix D. Bone mineral density in prepubertal obese and control children: relation to body weight, lean mass, and fat mass. Journal of bone and mineral metabolism. 2008;26(1):73-8.
113. Sioen I, Lust E, De Henauw S, Moreno LA, Jiménez-Pavón D. Associations Between Body Composition and Bone Health in Children and Adolescents: A Systematic Review. Calcif Tissue Int. 2016;99(6):557-77.
114. Farr JN, Dimitri P. The Impact of Fat and Obesity on Bone Microarchitecture and Strength in Children. Calcif Tissue Int. 2017;100(5):500-13.
115. Heydenreich J, Schweter A, Lührmann P. Association between Body Composition, Physical Activity, Food Intake and Bone Status in German Children and Adolescents. Int J Environ Res Public Health. 2020;17(19).
116. Forero-Bogotá MA, Ojeda-Pardo ML, García-Hermoso A, Correa-Bautista JE, González-Jiménez E, Schmidt-RíoValle J, et al. Body Composition, Nutritional Profile and Muscular Fitness Affect Bone Health in a Sample of Schoolchildren from Colombia: The Fuprecol Study. Nutrients. 2017;9(2).
117. Sioen I, Mouratidou T, Herrmann D, De Henauw S, Kaufman JM, Molnar D, et al. Relationship between markers of body fat and calcaneal bone stiffness differs between preschool and primary school children: results from the IDEFICS baseline survey. Calcif

Tissue Int. 2012;91(4):276-85.
118.Clark EM, Ness AR, Bishop NJ, Tobias JH. Association between bone mass and fractures in children: a prospective cohort study. Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research. 2006;21(9):1489-95.
119.Mikolajewicz N, Bishop N, Burghardt AJ, Folkestad L, Hall A, Kozloff KM, et al. HR-pQCT Measures of Bone Microarchitecture Predict Fracture: Systematic Review and Meta-Analysis. Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research. 2020;35(3):446-59.
120. Sakka SD, Cheung MS. Management of primary and secondary osteoporosis in children. Therapeutic advances in musculoskeletal disease. 2020;12:1759720x20969262.
121. Bonjour JP, Chevalley T, Ferrari S, Rizzoli R. The importance and relevance of peak bone mass in the prevalence of osteoporosis. Salud publica de Mexico. 2009;51 Suppl 1:S5-17.
122. Ahrens W, Bammann K, Siani A, Buchecker K, De Henauw S, Iacoviello L, et al. The IDEFICS cohort: design, characteristics and participation in the baseline survey. Int J Obes (Lond). 2011;35 Suppl 1:S3-15.
123. Ahrens W, Siani A, Adan R, De Henauw S, Eiben G, Gwozdz W, et al. Cohort Profile: The transition from childhood to adolescence in European children-how I.Family extends the IDEFICS cohort. Int J Epidemiol. 2017;46(5):1394-5j.
124. Stomfai S, Ahrens W, Bammann K, Kovács E, Mårild S, Michels N, et al. Intra- and inter-observer reliability in anthropometric measurements in children. Int J Obes (Lond). 2011;35 Suppl 1:S45-51.
125. Peplies J, Günther K, Bammann K, Fraterman A, Russo P, Veidebaum T, et al. Influence of sample collection and preanalytical sample processing on the analyses of biological markers in the European multicentre study IDEFICS. Int J Obes (Lond). 2011;35 Suppl 1:S104-12.
126. Herrmann D. Bone Stiffness and its Association with Physical Activity in Children : an Epidemiological Perspective. 2015.
127. Cole TJ, Freeman JV, Preece MA. British 1990 growth reference centiles for weight, height, body mass index and head circumference fitted by maximum penalized likelihood. Stat Med. 1998;17(4):407-29.
128. Cole TJ, Lobstein T. Extended international (IOTF) body mass index cut-offs for thinness, overweight and obesity. Pediatr Obes. 2012;7(4):284-94.
129. Rodríguez G, Moreno LA, Blay MG, Blay VA, Fleta J, Sarría A, et al. Body fat measurement in adolescents: comparison of skinfold thickness equations with dual-energy X-ray absorptiometry. Eur J Clin Nutr. 2005;59(10):1158-66.
130. Silva DR, Ribeiro AS, Pavão FH, Ronque ER, Avelar A, Silva AM, et al. Validity of the methods to assess body fat in children and adolescents using multi-compartment models as the reference method: a systematic review. Rev Assoc Med Bras (1992). 2013;59(5):475-86.
131. Nagy P, Kovacs E, Moreno LA, Veidebaum T, Tornaritis M, Kourides Y, et al. Percentile reference values for anthropometric body composition indices in European children from the IDEFICS study. Int J Obes (Lond). 2014;38 Suppl 2:S15-25.
132. Buck C, Eiben G, Lauria F, Konstabel K, Page A, Ahrens W, et al. Urban

Moveability and physical activity in children: longitudinal results from the IDEFICS and I.Family cohort. Int J Behav Nutr Phys Act. 2019;16(1):128.
133. Konstabel K, Veidebaum T, Verbestel V, Moreno LA, Bammann K, Tornaritis M, et al. Objectively measured physical activity in European children: the IDEFICS study. Int J Obes (Lond). 2014;38 Suppl 2:S135-43.
134. Evenson KR, Catellier DJ, Gill K, Ondrak KS, McMurray RG. Calibration of two objective measures of physical activity for children. Journal of sports sciences. 2008;26(14):1557-65.
135. Hirshkowitz M, Whiton K, Albert SM, Alessi C, Bruni O, DonCarlos L, et al. National Sleep Foundation's updated sleep duration recommendations: final report. Sleep Health. 2015;1(4):233-43.
136. Bel-Serrat S, Mouratidou T, Pala V, Huybrechts I, Börnhorst C, Fernández-Alvira JM, et al. Relative validity of the Children's Eating Habits Questionnaire-food frequency section among young European children: the IDEFICS Study. Public Health Nutr. 2014;17(2):266-76.
137. Huybrechts I, Börnhorst C, Pala V, Moreno LA, Barba G, Lissner L, et al. Evaluation of the Children's Eating Habits Questionnaire used in the IDEFICS study by relating urinary calcium and potassium to milk consumption frequencies among European children. Int J Obes (Lond). 2011;35 Suppl 1:S69-78.
138. Lanfer A, Hebestreit A, Ahrens W, Krogh V, Sieri S, Lissner L, et al. Reproducibility of food consumption frequencies derived from the Children's Eating Habits Questionnaire used in the IDEFICS study. Int J Obes (Lond). 2011;35 Suppl 1:S61-8.
139. Börnhorst C, Bel-Serrat S, Pigeot I, Huybrechts I, Ottavaere C, Sioen I, et al. Validity of 24-h recalls in (pre-)school aged children: comparison of proxy-reported energy intakes with measured energy expenditure. Clinical nutrition (Edinburgh, Scotland). 2014;33(1):79-84.
140. Suling M, Hebestreit A, Peplies J, Bammann K, Nappo A, Eiben G, et al. Design and results of the pretest of the IDEFICS study. Int J Obes (Lond). 2011;35 Suppl 1:S30-44.
141. Tooze JA, Midthune D, Dodd KW, Freedman LS, Krebs-Smith SM, Subar AF, et al. A new statistical method for estimating the usual intake of episodically consumed foods with application to their distribution. J Am Diet Assoc. 2006;106(10):1575-87.
142. Kipnis V, Midthune D, Buckman DW, Dodd KW, Guenther PM, Krebs-Smith SM, et al. Modeling data with excess zeros and measurement error: application to evaluating relationships between episodically consumed foods and health outcomes. Biometrics. 2009;65(4):1003-10.
143. Börnhorst C, Huybrechts I, Hebestreit A, Krogh V, De Decker A, Barba G, et al. Usual energy and macronutrient intakes in 2-9-year-old European children. Int J Obes (Lond). 2014;38 Suppl 2:S115-23.
144. EFSA Panel on Dietetic Products N, Allergies. Scientific opinion on dietary reference values for calcium. EFSA Journal. 2015;13(5):4101.
145. Statistics UIf. International standard classification of education: ISCED 2011: UNESCO Institute for Statistics Montreal; 2012.
146. Powers JM, Murphy JEJ. Sunlight radiation as a villain and hero: 60 years of illuminating research. International journal of radiation biology. 2019;95(7):1043-9.
147. Hagg U, Taranger J. Menarche and voice change as indicators of the pubertal growth

spurt. Acta Odontol Scand. 1980;38(3):179-86.
148. Iwaniec UT, Turner RT. Influence of body weight on bone mass, architecture and turnover. J Endocrinol. 2016;230(3):R115-30.
149. Thivel D, Ring-Dimitriou S, Weghuber D, Frelut ML, O'Malley G. Muscle Strength and Fitness in Pediatric Obesity: a Systematic Review from the European Childhood Obesity Group. Obesity facts. 2016;9(1):52-63.
150. Boye KR, Dimitriou T, Manz F, Schoenau E, Neu C, Wudy S, et al. Anthropometric assessment of muscularity during growth: estimating fat-free mass with 2 skinfold-thickness measurements is superior to measuring midupper arm muscle area in healthy prepubertal children. Am J Clin Nutr. 2002;76(3):628-32.
151. Perotti M, Perra S, Saluzzi A, Grassi G, Pincelli AI. Body fat mass is a strong and negative predictor of peak stimulated growth hormone and bone mineral density in healthy adolescents during transition period. Hormone and metabolic research = Hormon- und Stoffwechselforschung = Hormones et metabolisme. 2013;45(10):748-53.
152. Kindler JM, Lobene AJ, Vogel KA, Martin BR, McCabe LD, Peacock M, et al. Adiposity, Insulin Resistance, and Bone Mass in Children and Adolescents. J Clin Endocrinol Metab. 2019;104(3):892-9.
153. Kindler JM, Pollock NK, Laing EM, Oshri A, Jenkins NT, Isales CM, et al. Insulin Resistance and the IGF-I-Cortical Bone Relationship in Children Ages 9 to 13 Years. Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research. 2017;32(7):1537-45.
154. Dimitri P, Bishop N, Walsh JS, Eastell R. Obesity is a risk factor for fracture in children but is protective against fracture in adults: a paradox. Bone. 2012;50(2):457-66.
155. Burt Solorzano CM, McCartney CR. Obesity and the pubertal transition in girls and boys. Reproduction (Cambridge, England). 2010;140(3):399-410.
156. Dimitri P, Jacques RM, Paggiosi M, King D, Walsh J, Taylor ZA, et al. Leptin may play a role in bone microstructural alterations in obese children. J Clin Endocrinol Metab. 2015;100(2):594-602.
157. Dimitri P, Wales JK, Bishop N. Adipokines, bone-derived factors and bone turnover in obese children; evidence for altered fat-bone signalling resulting in reduced bone mass. Bone. 2011;48(2):189-96.
158. Upadhyay J, Farr OM, Mantzoros CS. The role of leptin in regulating bone metabolism. Metabolism: clinical and experimental. 2015;64(1):105-13.
159. LeBlanc AG, Katzmarzyk PT, Barreira TV, Broyles ST, Chaput JP, Church TS, et al. Correlates of Total Sedentary Time and Screen Time in 9-11 Year-Old Children around the World: The International Study of Childhood Obesity, Lifestyle and the Environment. PloS one. 2015;10(6):e0129622.
160. Pelegrini A, Klen JA, Costa AM, Bim MA, Claumann GS, De Angelo HCC, et al. Association between sedentary behavior and bone mass in adolescents. Osteoporos Int. 2020;31(9):1733-40.
161. Robling AG, Burr DB, Turner CH. Partitioning a daily mechanical stimulus into discrete loading bouts improves the osteogenic response to loading. Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research. 2000;15(8):1596-602.

162. Robling AG, Hinant FM, Burr DB, Turner CH. Improved bone structure and strength after long-term mechanical loading is greatest if loading is separated into short bouts. Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research. 2002;17(8):1545-54.
163. Chastin SF, Mandrichenko O, Skelton DA. The frequency of osteogenic activities and the pattern of intermittence between periods of physical activity and sedentary behaviour affects bone mineral content: the cross-sectional NHANES study. BMC public health. 2014;14:4.
164. Herrmann D, Pohlabeln H, Gianfagna F, Konstabel K, Lissner L, Mårild S, et al. Association between bone stiffness and nutritional biomarkers combined with weight-bearing exercise, physical activity, and sedentary time in preadolescent children. A case-control study. Bone. 2015;78:142-9.
165. McCormack L, Meendering J, Specker B, Binkley T. Associations Between Sedentary Time, Physical Activity, and Dual-Energy X-ray Absorptiometry Measures of Total Body, Android, and Gynoid Fat Mass in Children. J Clin Densitom. 2016;19(3):368-74.
166. Sioen I, Michels N, Polfliet C, De Smet S, D'Haese S, Roggen I, et al. The influence of dairy consumption, sedentary behaviour and physical activity on bone mass in Flemish children: a cross-sectional study. BMC public health. 2015;15:717.
167. Ivuškāns A, Mäestu J, Jürimäe T, Lätt E, Purge P, Saar M, et al. Sedentary time has a negative influence on bone mineral parameters in peripubertal boys: a 1-year prospective study. Journal of bone and mineral metabolism. 2015;33(1):85-92.
168. Kohrt WM, Bloomfield SA, Little KD, Nelson ME, Yingling VR. American College of Sports Medicine Position Stand: physical activity and bone health. Med Sci Sports Exerc. 2004;36(11):1985-96.
169. Richmond E, Rogol AD. Endocrine Responses to Exercise in the Developing Child and Adolescent. Frontiers of hormone research. 2016;47:58-67.
170. Kish K, Mezil Y, Ward WE, Klentrou P, Falk B. Effects of plyometric exercise session on markers of bone turnover in boys and young men. Eur J Appl Physiol. 2015;115(10):2115-24.
171. Maïmoun L, Sultan C. Effects of physical activity on bone remodeling. Metabolism: clinical and experimental. 2011;60(3):373-88.
172. Börnhorst C, Hense S, Ahrens W, Hebestreit A, Reisch L, Barba G, et al. From sleep duration to childhood obesity--what are the pathways? Eur J Pediatr. 2012;171(7):1029-38.
173. Thumann BF, Michels N, Felső R, Hunsberger M, Kaprio J, Moreno LA, et al. Associations between sleep duration and insulin resistance in European children and adolescents considering the mediating role of abdominal obesity. PloS one. 2020;15(6):e0235049.
174. Sparano S, Lauria F, Ahrens W, Fraterman A, Thumann B, Iacoviello L, et al. Sleep duration and blood pressure in children: Analysis of the pan-European IDEFICS cohort. J Clin Hypertens (Greenwich). 2019;21(5):572-8.
175. Mindell JA, Williamson AA. Benefits of a bedtime routine in young children: Sleep, development, and beyond. Sleep Med Rev. 2018;40:93-108.
176. Morrissey B, Taveras E, Allender S, Strugnell C. Sleep and obesity among children: A systematic review of multiple sleep dimensions. Pediatr Obes. 2020;15(4):e12619.

177. Swanson CM, Kohrt WM, Buxton OM, Everson CA, Wright KP, Jr., Orwoll ES, et al. The importance of the circadian system & sleep for bone health. Metabolism: clinical and experimental. 2018;84:28-43.
178. Morris CJ, Aeschbach D, Scheer FA. Circadian system, sleep and endocrinology. Mol Cell Endocrinol. 2012;349(1):91-104.
179. Perrini S, Laviola L, Carreira MC, Cignarelli A, Natalicchio A, Giorgino F. The GH/IGF1 axis and signaling pathways in the muscle and bone: mechanisms underlying age-related skeletal muscle wasting and osteoporosis. J Endocrinol. 2010;205(3):201-10.
180. Irwin MR, Olmstead R, Carroll JE. Sleep Disturbance, Sleep Duration, and Inflammation: A Systematic Review and Meta-Analysis of Cohort Studies and Experimental Sleep Deprivation. Biol Psychiatry. 2016;80(1):40-52.
181. Dumuid D, Simm P, Wake M, Burgner D, Juonala M, Wu F, et al. The "Goldilocks Day" for Children's Skeletal Health: Compositional Data Analysis of 24-Hour Activity Behaviors. Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research. 2020;35(12):2393-403.
182. Rollo S, Antsygina O, Tremblay MS. The whole day matters: Understanding 24-hour movement guideline adherence and relationships with health indicators across the lifespan. Journal of sport and health science. 2020;9(6):493-510.
183. Constable AM, Vlachopoulos D, Barker AR, Moore SA, Soininen S, Haapala EA, et al. The independent and interactive associations of physical activity intensity and vitamin D status with bone mineral density in prepubertal children: the PANIC Study. Osteoporos Int. 2021.
184. Abrams SA, Griffin IJ, Hawthorne KM, Gunn SK, Gundberg CM, Carpenter TO. Relationships among vitamin D levels, parathyroid hormone, and calcium absorption in young adolescents. J Clin Endocrinol Metab. 2005;90(10):5576-81.
185. DeBoer MD, Weber DR, Zemel BS, Denburg MR, Herskovitz R, Long J, et al. Bone Mineral Accrual Is Associated With Parathyroid Hormone and 1,25-Dihydroxyvitamin D Levels in Children and Adolescents. J Clin Endocrinol Metab. 2015;100(10):3814-21.
186. Lee JH, Ha AW, Kim WK, Kim SH. The Combined Effects of Milk Intake and Physical Activity on Bone Mineral Density in Korean Adolescents. Nutrients. 2021;13(3).
187. Yang X, Zhai Y, Zhang J, Chen JY, Liu D, Zhao WH. Combined effects of physical activity and calcium on bone health in children and adolescents: a systematic review of randomized controlled trials. World journal of pediatrics : WJP. 2020;16(4):356-65.
188. Duquette J, Lin J, Hoffman A, Houde J, Ahmadi S, Baran D. Correlations among bone mineral density, broadband ultrasound attenuation, mechanical indentation testing, and bone orientation in bovine femoral neck samples. Calcif Tissue Int. 1997;60(2):181-6.
189. Lenora J, Gerdhem P, Obrant KJ, Ivaska KK. Bone turnover markers are correlated with quantitative ultrasound of the calcaneus: 5-year longitudinal data. Osteoporos Int. 2009;20(7):1225-32.
190. Nishimura T, Arima K, Abe Y, Kanagae M, Mizukami S, Okabe T, et al. Relationship between bone turnover markers and the heel stiffness index measured by quantitative ultrasound in middle-aged and elderly Japanese men. Medicine. 2018;97(8):e9962.

Appendix

Paper 1

Sex differences in the longitudinal associations between body composition and bone stiffness index in European children and adolescents

Lan Cheng, Hermann Pohlabeln, Wolfgang Ahrens, Paola Russo, Toomas Veidebaum, Charalambos Chadjigeorgiou, Dénes Molnár, Gabriele Eiben, Stefaan De Henauw, Luis Moreno, Angie Page, Antje Hebestreit, on behalf of the IDEFICS and I.Family Consortia

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Sex differences in the longitudinal associations between body composition and bone stiffness index in European children and adolescents

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Abbreviations

BMI: body mass index

FM: fat mass

FFM: fat free mass

BF: body fat

SI: stiffness index

QUS: quantitative ultrasound

BUA: broadband ultrasound attenuation

BMD: bone mineral density

DXA: dual-energy X-ray absorptiometry

pQCT: peripheral quantitative computed tomography

SOS: speed of sound

CV_{RMS}: root-mean-square coefficient of variation

BIA: bioelectrical impedance analysis

SES: family socioeconomic status

ISCED: International Standard Classification of Education

SB: sedentary behaviors

PA: physical activity

SD: standard deviation

CI: confidence interval

BMC: bone mineral content

CRP: C-reactive protein

Abstract

Fat mass (FM) and fat free mass (FFM) may influence bone health differentially. However, existing evidences on associations between FM, FFM and bone health are inconsistent and vary according to sex and maturity. The present study aims to evaluate longitudinal associations between FM, FFM and bone stiffness index (SI) among European children and adolescents with 6 years follow-up. A sample of 2468 children from the IDEFICS/I.Family was included, with repeated measurements of SI using calcaneal quantitative ultrasound, body composition using skinfold thickness, sedentary behaviors and physical activity using self-administrated questionnaires. Regression coefficients (β) and 99%-confidence intervals (99%CI) were calculated by sex-specified generalized linear mixed effects models to analyze the longitudinal associations between FM and FFM z-scores (zFM and zFFM) and SI percentiles, and to explore the possible interactions between zFM, zFFM and maturity. Baseline zFFM was observed to predict the change in SI percentiles in both boys (β= 4.57, 99%CI: 1.36, 7.78) and girls (β= 3.42, 99%CI: 0.05, 6.79) after 2 years. Moreover, baseline zFFM (β= 8.72, 99%CI: 3.18, 14.27 in boys and β= 5.89, 99%CI: 0.34, 11.44 in girls) and the change in zFFM (β= 6.58, 99%CI: 0.83, 12.34 in boys and β= 4.81, 99%CI: -0.41, 10.02 in girls) were positively associated with the change in SI percentiles after 6 years. In contrast, a negative association was observed between the change in zFM and SI percentiles in boys after 6 years (β= -3.70, 99%CI: -6.99, -0.42). Besides, an interaction was observed between the change in zFM and menarche on the change in SI percentiles in girls at 6 years follow-up (p= 0.009), suggesting a negative association before menarche while a positive association after menarche. Our findings support the existing evidences for a positive relationship between FFM and SI during growth. Furthermore, long-term FM gain was inversely associated with SI in boys, whereas opposing associations were observed across menarche in girls.

Keywords Pediatrics, body composition, bone stiffness index, sex differences, longitudinal study

1 Introduction

Effects of body composition on bone development are of increasing interest recently. In adulthood, adiposity serves as a protective factor against osteoporotic fractures [1, 2], whereas studies investigating the effect of adiposity on bone growth in children and adolescents still appear to be diverse [3]. In previous pediatric studies, the most widely used category of excess adiposity is body mass index (BMI). However, BMI cannot distinguish between fat mass (FM) and fat free mass (FFM), which are contributors to weight status and both of them have a mechanical loading on bone growth. Meanwhile FM may have both negative and positive effects on bone mass accrual mediated via endocrine pathways [4]. Childhood and adolescence present a particularly critical stage for bone growth, understanding the impact of body composition on bone health is important when planning prevention strategies towards fracture and osteoporosis in later life.

The effect of FM on bone strength is considered controversial, diverging results were reported in different sex, age and pubertal groups. For example, positive associations between FM and bone strength were observed in some studies among younger children, i.e. infants and pre-school children [5-7]. In contrast, Pollock et al. [8] found that percentage of body fat (BF, %) was inversely related to bone strength indexes in late adolescent females. However, Farr et al. [9] observed that total body FM was not cross-sectional associated with all bone strength parameters, while positively associated with 2 years changes of bone strength and density at weight-bearing site in 8 to 13 years old girls [10]. For now, the role of FM on bone strength during growth remains unclear.

The acquisition of bone strength is significantly influenced by the muscle function, and the positive associations between the muscle parameters (e.g. FFM, lean mass, skeletal muscle mass etc.) and bone strength have already been demonstrated in various cross-sectional [11, 12] and prospective studies [13]. In the IDEFICS study (Identification and prevention of dietary- and lifestyle induced health effects in children and infants) with children from 2 to 9 years old, muscular fitness and FFM were found to be positively associated with bone stiffness index (SI) measured using calcaneal

quantitative ultrasound (QUS) [14]. Another cross-sectional study among adolescents reported positive associations between lean mass and other QUS parameters such as broadband ultrasound attenuation (BUA) [15]. However, the associations between FFM and bone strength may also be sex-dependent during growth [16], and few studies have reported the association of FM on bone strength in children and adolescents while taking FFM into consideration. Some studies found that the associations among the FM and bone mass might be attenuated even reversed after adjusting for lean mass [9, 17, 18]. We also previously demonstrated in the IDEFICS study that primary school children with higher BF had higher calcaneal SI, but after adjusting for FFM, this relationship turned to be inverse [19]. Therefore, it is still important to clarify the independent effects of FFM and FM on bone strength development.

Some sophisticated methods such as dual-energy X-ray absorptiometry (DXA) and peripheral quantitative computed tomography (pQCT) have been shown to be useful measurements for bone parameters. However, these methods appear to be not suitable for large-scale studies among healthy children and adolescents. In this context, QUS as an alternative method was applied in the IDEFICS/I.Family study, which is gaining popularity because of its quick, cheap and non-radiating characteristics [20-22]. Calcaneal QUS has shown good correlation with DXA measurements [22] and has been suggested as an important indicator in determining fracture risk in adults [21, 23]. Previous studies that compared calcaneal SI with bone mineral density (BMD) measured by DXA of whole body, lumbar spine and hip among children and adolescents also reported significant correlation coefficients range from 0.5 to 0.7 [24-26]. Besides, QUS measurements provided good precision for the risk of osteopenia in young patients [24]. To our best knowledge, there are no studies addressing the role of body composition on QUS measured bone parameters in children and adolescents age from 2 to 15 years old using a large longitudinal multi-country cohort. In order to extend the current understanding between body composition and bone strength during growth, we

aimed to conduct a prospective analysis to evaluate relationship of changes in FM, FFM and SI, and to estimate whether these associations differ from sex and maturity.

2 Materials and Methods

2.1 Study sample

Data for the present longitudinal investigation was obtained from the IDEFICS/I.Family studies. Briefly, the aim of the IDEFICS study was to investigate dietary and behavioral disorders in young children, mainly focusing on overweight and obesity. The baseline data were collected within 16229 children aged 2 to 9.9 years old between September 2007 and June 2008 in eight European countries (Belgium, Cyprus, Estonia, Germany, Hungary, Italy, Spain and Sweden). The first follow-up examinations were performed between September 2009 and June 2010. The further follow-up examinations were performed between January 2013 and June 2014 in context of the I.Family study including 7117 children from the original IDEFICS cohort, to further explore the familial characteristics related to children's health development. All the examinations were conducted according to the Declaration of Helsinki. Parents gave written informed consent prior to study participation and children gave oral or signed simplified consent prior to the examinations. All participating centres have obtained ethical approval from the regional committees. Other details regarding study design have been published previously [27, 28].

As an optional examination module, approximate 50% of children in the IDEFICS study participated in the calcaneal QUS examination, and 5 of 8 participating countries with approximate 30% of children and adolescents in the I.Family study participated. In the present longitudinal analyses, 3422 participants who had baseline and at least one follow-up QUS measurements were included. According to a previous reliability study which compared the SI measurements across QUS devices among a convenience sample (N=91), a significant discrepancy was observed between the devices for the absolute SI difference of the left and the right foot (unpublished data). Hence, we excluded the sample with absolute difference of SI value above 97th percentile (41 unit) between the right and

left foot to control the discrepancy (N=200). Moreover, parents were asked to report a health and medical history questionnaire, whose answers were used to exclude children from the analysis with a history of medical condition known to affect bone metabolism or limit physical exercise (N=43) [14]. Furthermore, 711 participants had to be excluded because of incomplete data of body composition or covariates, leaving a total of 2468 children for the final analysis. Given these restrictions, no children from Cyprus remained in the final analysis sample.

2.2 Bone SI

The calcaneal QUS was used in the IDEFICS/I.Family cohort with Achilles Lunar Insight TM (GE Healthcare, Milwaukee, WI, USA), which had previously been described in details [19]. The calcaneus, as a weight-bearing skeletal site and consists of 90% trabecular bone, is the most common used measuring site. Two parameters were measured by calcaneal QUS: broadband ultrasound attenuation (BUA, dB/MHz), which represents the spatial orientation of the bone trabeculae and increases with greater trabecular complexity; the speed of sound (SOS, m/s), which represents the velocity of sound traveling through the bone and increases with greater structures density [29]. SI was automatically calculated from BUA and SOS by the device and expressed as 'unit' according to the equation: SI = (0.67*BUA) + (0.28*SOS) – 420. Measurements were performed by trained nurses following standardized procedures, two adaptors were used for different foot size. Both of the left and right feet were measured once at each of three time points. According to a previous reliability study which examined the reproducibility of SI measurements among 60 children from the baseline survey of the IDEFICS study, the root-mean-square coefficient of variation (CV_{RMS}) on the left foot and right foot were 7.2% and 9.2%, respectively. Besides, no significant difference was observed of repeated SI measurements compared to the first measurement in children (unpublished data). In the present study, the mean of two SI for each foot was used for analysis. For each individual, the SI percentiles were calculated additionally according to age, sex and height based on the IDEFICS/I.Family reference population [30].

2.3 Body composition

FM (kg) was estimated by skinfold thickness based on Slaughter's equations, which are most commonly used for population-based studies in children and adolescents, and showed the reliable results for the assessment of BF [31, 32]. FFM (kg) was used as an indicator of skeletal muscle mass, which was calculated by the equation FFM = Body weight - FM. The Tanita scales (BC420 MA for children and BC418 MA for adolescents, TANITA Europe GmbH, Sindelfingen, Germany) were used to measure body weight (kg) to the nearest 0.1 kg in light clothes without shoes. Skinfold thickness (mm) was measured at subscapular and triceps according to the international standards for anthropometric assessment [33]. Subscapular was measured about 20 mm below the tip of the scapula, at an angle of 45° to the lateral side of the body, and triceps was measured halfway between the acromion and the olecranon process at the back of the arm. Measurements were obtained twice at each site to the nearest 0.2 mm with a skinfold calliper (Holtain, Crosswell, UK; range 0–40 mm). The mean of the two measurements was calculated and used for later analyses. All the measurements were performed by well-trained field staffs, standard operation procedures were pre-tested in each participating centre for their feasibility and acceptability before the baseline survey [34]. The intra and inter- observer reliability of skinfold thickness was considered within an acceptable range in the IDEFICS [33] and I.Family validation studies (unpublished data). In the present analysis, FM and FFM age- and sex-specific z-scores (zFM and zFFM) were derived based on the IDEFICS/I.Family reference population [35].

In the exploratory phase of the study, the indicators of FM and FFM measured using the Tanita scales were also taken into consideration. However, according to the IDEFICS validation study, the explained variances of skinfold measurement were found to be slightly higher than the bioelectrical impedance analysis (BIA) [36, 37]. Besides, in a subsample of young obese children, skinfold estimate rather than BIA estimate was found to be positively correlated with BF(%) measured using

DXA and BodPod (unpublished results). Hence this part of results was not included in the final analyses.

2.4 Self-assessment maturational status

Menarche in girls and voice change in boys were used as indicators of maturation [38], which have been found to occur around Tanners pubertal stages 3 and 4 [39, 40]. Menarcheal age is widely used in epidemiological studies to provide sexual maturational information in female [41], and voice change as a proxy for male maturity has been related to anthropometric growth [42]. In the I.Family study, boys and girls above 8 years old were instructed either by the study nurse or physician to self-report their maturity using a sex-appropriate one-page questionnaire.

2.5 Confounding variables

The age and sex of children as well as family socioeconomic status (SES) were obtained by one of the parents from a self-administered proxy-questionnaire. SES was estimated by the maximum of parental education based on International Standard Classification of Education (ISCED), levels 0 to 2 were defined as low and level 3 to 4 were defined as medium while level 5 and 6 were defined as high [43]. In addition, parents of children up to 11 years and 12 to 15 years old adolescents completed a questionnaire to assess sedentary behaviors (SB) and physical activity (PA) of the child, by reporting the weekly duration of total screen time (including watching/TV/videos/DVDs and playing computer/game) and participating in sports clubs. Height (cm) was measured by stadiometer (SECA 225, Seca GmbH & KG, Birmingham, UK) to the nearest 0.1 cm without shoes, and age- and sex- specific z-scores of height were calculated using the LMS method by Cole [44]. Considering the sun exposure is associated with vitamin D synthesis and further may influence the bone mass accrual [45], we calculated mean daylight duration (±0.1 h) at baseline for each examination month in each location using astronomical tables [14], as a proxy for the child exposure to sunlight.

2.6 Statistical analyses

All statistical calculations were performed using SAS software (V9.3; SAS Institute Inc, Cary, North Carolina, USA). Simple descriptive statistics (means, standard deviations (SD), and frequencies) at baseline and twice follow-up were presented by cross-classified tables, stratified by sex. The changes of all dependent and independent variables were calculated as the within-individual difference between a follow-up measurement and the corresponding baseline measurement.

Sex-specific generalized linear mixed effects models were used to analyze the longitudinal relationship between body composition and SI percentiles, with country as a random effect (at the level of the intercept) to take into account the cluster sampling design. Meanwhile, age (continuous variable), SES, daylight duration, SI percentiles and height z-score at baseline as well as change in height z-score were included as fixed terms, while maturational status was only available at 6 years follow-up. The outcomes were changes in SI percentiles after 2 years and 6 years, and the exposures in terms of zFM and zFFM were considered as both baseline covariates and change covariates. In model 1 and model 2, zFM and zFFM were included in the models separately, and then were included simultaneously in model 3. In order to further explore whether the associations between body composition z-scores and SI percentiles were influenced by SB and PA, we additionally adjusted for the average duration of SB and PA in model 4, which were derived from the means of baseline and corresponding follow-up value. In all models, means of parameter estimates (β) and 99%-confidence intervals (99%CI) were calculated. To avoid that meaningless associations become statistically significant (just because of the large sample size), we carried out multiple tests of associations with choosing a more stringent criterion for statistical significance ($\alpha = 0.01$).

Moreover, interaction effects were analyzed between body composition and maturity based on model 3. Possible interactive effects were stratified by maturity when statistically significant.

3 Results

3.1 Baseline and follow-up descriptive characteristics

Among 2468 participants who were included in the study, 1274 (51.6%) were boys, and the average age in boys and girls were 6.23 and 6.39, respectively. Of these, 2144 individuals provided full information after 2 years and 833 individuals provided after 6 years. 42.5% of boys reported having voice change and 40.1% of girls reported having first menstrual period at 6 years follow-up (Table 1). We further conducted the attrition analysis regarding main demographic characteristics (i.e. sex, age and family SES) between the participants who were included in each follow-up analytic sample and the non-participants who took part in the QUS module at baseline but didn't provide follow-up and/or complete co-variate information (N=5071). Overall, there was no significant difference for sex and SES after 2 years, while the participants (6.44±1.69) were older than the non-participants (6.00±1.82, $p< 0.001$). Meanwhile, no significant difference was found for sex and age after 6 years, while more participants were defined as low (10.4%) and medium level SES (67.2%) than the non-participants (9.2% and 51.0%, respectively, $p< 0.001$).

At baseline, the mean SI was 78.09 ± 12.41 in boys and 77.24 ± 12.86 in girls. Boys had a slightly higher FFM (19.74 ± 4.74) while lower FM (4.14 ± 2.94) compared to girls (19.20 ± 4.53 and 4.73 ± 3.03, respectively). The mean height of boys (119.40 ± 12.55) and girls (119.40 ± 12.50) were similar. All the anthropometric measurements showed comparable increasing trends in both sexes over 2 years and 6 years periods (Table 1).

Table 1. Descriptive characteristics for participants at baseline, 2 years and 6 years follow-up, stratified by sex

	Baseline (N=2468)		2 years follow-up (N=2144)		6 years follow-up (N=833)	
	Boys N=1274	Girls N=1194	Boys N=1112	Girls N=1032	Boys N=402	Girls N=431
Age (Mean, SD)	6.23(1.75)	6.39(1.75)	8.35(1.70)	8.50(1.68)	11.9(1.78)	12.0(1.79)
Socioeconomic status (N, %)						
Low	110(8.6)	119(10.0)	89(8.0)	101(9.8)	42(10.5)	45(10.4)
Medium	742(58.2)	639(53.5)	630(56.7)	530(51.4)	275(68.4)	285(66.1)
High	422(33.1)	436(36.5)	393(35.3)	401(38.9)	85(21.1)	101(23.4)
Maturational status (N, %)*						
Pre- or early mature	/	/	/	/	231(57.5)	258(59.9)
Mature	/	/	/	/	171(42.5)	173(40.1)
Country (N, %)						
Belgium	176(13.8)	139(11.6)	176(15.8)	139(13.5)	/	/
Estonia	222(17.4)	202(16.9)	154(13.9)	135(13.1)	126(31.3)	121(28.1)
Germany	356(27.9)	352(29.5)	298(26.8)	285(27.6)	143(35.6)	180(41.8)
Hungary	86(6.8)	78(6.5)	86(7.7)	78(7.6)	/	/
Italy	244(19.2)	231(19.4)	218(19.6)	212(20.5)	100(24.9)	96(22.3)
Spain	90(7.1)	85(7.1)	80(7.2)	76(7.4)	33(8.2)	34(7.9)
Sweden	100(7.9)	107(9.0)	100(9.0)	107(10.4)	/	/
Anthropometric measurements (Mean, SD)						
Bone stiffness index	78.09(12.41)	77.24(12.86)	82.24(13.46)	82.14(12.95)	88.88(14.56)	90.26(15.76)
Bone stiffness index percentiles	43.19(27.50)	42.05(26.80)	46.32(28.81)	47.21(28.04)	50.39(28.11)	53.34(28.21)
Fat free mass (kg)	19.74(4.74)	19.20(4.53)	24.63(5.23)	24.03(5.20)	36.61(8.91)	35.13(7.83)
Fat free mass z-score	0.23(1.25)	0.25(1.27)	0.25(1.19)	0.19(1.27)	0.18(1.09)	0.25(1.25)
Fat mass (kg)	4.14(2.94)	4.73(3.03)	6.19(5.07)	6.90(4.49)	10.78(7.64)	12.14(6.94)
Fat mass z-score	0.33(1.48)	0.48(1.58)	0.42(1.53)	0.56(1.66)	0.72(1.39)	0.69(1.56)
Height (cm)	119.40 (12.55)	119.40(12.50)	132.60(11.42)	132.60(11.36)	154.1(12.42)	153.0(11.48)
Height z-score	0.54(1.03)	0.45(1.04)	0.58(1.05)	0.49(1.00)	0.69(1.00)	0.58(1.11)
Reported healthy behaviors (Mean, SD)						
Duration of screen time (hours/week)	12.17(7.47)	10.62(6.38)	14.52(7.83)	12.60(6.96)	19.85(11.80)	13.98(8.56)
Duration of sports clubs (hours/week)	1.33(1.66)	1.39(1.77)	2.09(1.92)	2.10(2.25)	2.73(2.38)	2.41(2.72)

** Menarche in girls and voice change in boys were used as indicators of maturation*

3.2 Longitudinal effects of body composition z-scores on changes in SI percentiles

As presented in table 2, the baseline zFFM positively predicted the change in SI percentiles in both boys and girls after 2 years (Model 1), these positive associations persisted after adjustments of baseline and change in zFM in model 3 (β= 4.57, 99%CI: 1.36, 7.78 in boys and β= 3.42, 99%CI: 0.05, 6.79 in girls, respectively). Meanwhile, the baseline zFM tended to be positively related to change in SI percentiles in both sexes (Model 2), these associations were reversed, however still not statistically significant, after taking zFFM into consideration in model 3 (β= -1.18, 99%CI: -3.02, 0.66 in boys and β= -0.14, 99%CI: -2.00, 1.72 in girls, respectively). Additional adjustments of SB and PA in model 4 only resulted in a slight decrease in the effect sizes compared to model 3, whereas remained nearly unchanged.

In table 3, the baseline zFFM was also observed to positively predict the changes in SI percentiles in both sexes after 6 years (model 1). These results were also valid when additionally adjusted for zFM in model 3 (β= 8.72, 99%CI: 3.18, 14.27 in boys and β= 5.89, 99%CI: 0.34, 11.44 in girls). Besides, a positive association between changes in zFFM and SI percentiles were also observed in boys (β= 6.58, 99%CI: 0.83, 12.34), similar but not statistically significant association also can be seen in girls (β= 4.81, 99%CI: -0.41, 10.02) in model 3. Likewise, additionally adjusting for SB and PA in model 4 nearly did not change these associations. On the contrary, the positive effect of baseline zFM on change in SI percentiles in model 2 was attenuated in girls (β= 1.42, 99%CI: -1.72, 4.56) and reversed in boys (β= -0.20, 99%CI: -3.55, 3.14) after adjusting for zFFM in model 3. Moreover, a negative association between change in zFM and SI percentiles was observed in boys (β= -3.70, 99%CI: -6.99, -0.42), whereas the effect estimate decreased and became statistically insignificant in model 4.

Table 2. Associations between body composition z-scores and change in bone stiffness index percentiles after 2 years, stratified by sex

	Model 1[a]		Model 2[b]		Model 3[c]		Model 4[d]	
	β (99%CI)	p-value	β (99%CI)	p-value	β (99%CI)	p-value	β (99%CI)	p-value
Boys (N=1112)								
Baseline fat free mass z-score	3.44(0.69,6.19)	0.001	/	/	4.57(1.36,7.78)	<0.001	4.35(1.14,7.56)	0.001
Change in fat free mass z-score	2.11(-2.05,6.28)	0.191	/	/	2.94(-1.35,7.24)	0.077	2.78(-1.52,7.07)	0.096
Baseline fat mass z-score	/	/	0.14(-1.43,1.72)	0.812	-1.18(-3.02,0.66)	0.097	-1.06(-2.90,0.77)	0.136
Change in fat mass z-score	/	/	-1.02(-3.63,1.58)	0.311	-2.00(-4.71,0.71)	0.057	-1.89(-4.60,0.82)	0.073
Average duration of screen time (hours/week)	/	/	/	/	/	/	0.01(-0.29,0.31)	0.954
Average duration of sports clubs (hours/week)	/	/	/	/	/	/	1.23(-0.12,2.59)	0.019
Girls (N=1032)								
Baseline fat free mass z-score	3.21(0.76,5.66)	0.001	/	/	3.42(0.05,6.79)	0.009	3.19(-0.17,6.56)	0.015
Change in fat free mass z-score	1.35(-2.58,5.27)	0.377	/	/	1.65(-2.47,5.76)	0.302	1.47(-2.63,5.58)	0.354
Baseline fat mass z-score	/	/	1.15(-0.21,2.51)	0.029	-0.14(-2.00,1.72)	0.847	0.05(-1.82,1.91)	0.95
Change in fat mass z-score	/	/	-0.15(-2.60,2.30)	0.875	-0.87(-3.44,1.70)	0.383	-0.60(-3.18,1.99)	0.552
Average duration of screen time (hours/week)	/	/	/	/	/	/	-0.14(-0.47,0.19)	0.283
Average duration of sports clubs (hours/week)	/	/	/	/	/	/	0.97(-0.10,2.05)	0.019

All the models were adjusted for age, socioeconomic status, daylight, bone stiffness index percentiles and height z-score at baseline as well as change in height z-score, country as a random effect

a Model 1 only included baseline and change in fat free mass z-score as exposures; b Model 2 only included baseline and change in fat mass z-score as exposures; c Model 3 included baseline and change in fat free mass z-score as well as fat mass z-score to test their independent associations with bone stiffness index percentiles; d Model 4 was Model 3 additionally adjusted for average duration of screen time and sports clubs

Table 3. Associations between body composition z-scores and change in bone stiffness index percentiles after 6 years, stratified by sex

	Model 1[a]		Model 2[b]		Model 3[c]		Model 4[d]	
	β (99%CI)	p-value	β (99%CI)	p-value	β (99%CI)	p-value	β (99%CI)	p-value
Boys (N=402)								
Baseline fat free mass z-score	9.21(4.30,14.11)	<0.001	/	/	8.72(3.18,14.27)	<0.001	8.15(2.62,13.67)	<0.001
Change in fat free mass z-score	5.52(-0.24,11.28)	0.014	/	/	6.58(0.83,12.34)	0.003	5.93(0.21,11.66)	0.008
Baseline fat mass z-score	/	/	1.95(-0.99,4.90)	0.087	-0.20(-3.55,3.14)	0.874	0.11(-3.20,3.42)	0.932
Change in fat mass z-score	/	/	-2.93(-6.25,0.39)	0.023	-3.70(-6.99,-0.42)	0.004	-2.99(-6.29,0.31)	0.02
Average duration of screen time (hours/week)	/	/	/	/	/	/	-0.11(-0.56,0.34)	0.53
Average duration of sport clubs (hours/week)	/	/	/	/	/	/	2.56(0.43,4.69)	0.002
Girls (N=431)								
Baseline fat free mass z-score	7.61(3.67,11.54)	<0.001	/	/	5.89(0.34,11.44)	0.006	5.15(-0.36,10.67)	0.016
Change in fat free mass z-score	4.49(-0.28,9.26)	0.015	/	/	4.81(-0.41,10.02)	0.018	5.34(0.05,10.63)	0.009
Baseline fat mass z-score	/	/	3.62(1.38,5.86)	<0.001	1.42(-1.72,4.56)	0.243	1.99(-1.14,5.12)	0.1
Change in fat mass z-score	/	/	1.47(-1.39,4.33)	0.185	-0.30(-3.49,2.89)	0.809	0.02(-3.14,3.17)	0.988
Average duration of screen time (hours/week)	/	/	/	/	/	/	-0.23(-0.77,0.31)	0.268
Average duration of sport clubs (hours/week)	/	/	/	/	/	/	2.04(0.39,3.69)	0.002

All the models were adjusted for age, socioeconomic status, daylight, bone stiffness index percentiles and height z-score at baseline, as well as change in height z-score and maturity after 6 years, country as a random effect

a Model 1 only included baseline and change in fat free mass z-score as exposures; b Model 2 only included baseline and change in fat mass z-score as exposures; c Model 3 included baseline and change in fat free mass z-score as well as fat mass z-score to test their independent associations with bone stiffness index percentiles; d Model 4 was Model 3 additionally adjusted for average duration of screen time and sports clubs

3.3 Interactive effects between body composition z-scores and maturational status on changes in SI percentiles

Different estimates were found in girls when investigating possible interactions, resulting in a p-value of 0.009 for the interaction term of change in zFM and menarche. A negative association between change in zFM and SI percentiles was observed in girls before menarche (β= -1.81, 99%CI: -5.91, 2.30, p= 0.254), whereas a positive association was observed in girls after menarche (β= 3.46, 99%CI: -1.55, 8.47, p= 0.074), these associations however were not statistically significant (Fig. 1). Besides, tests for interaction between body composition and voice change among boys were not statistically significant.

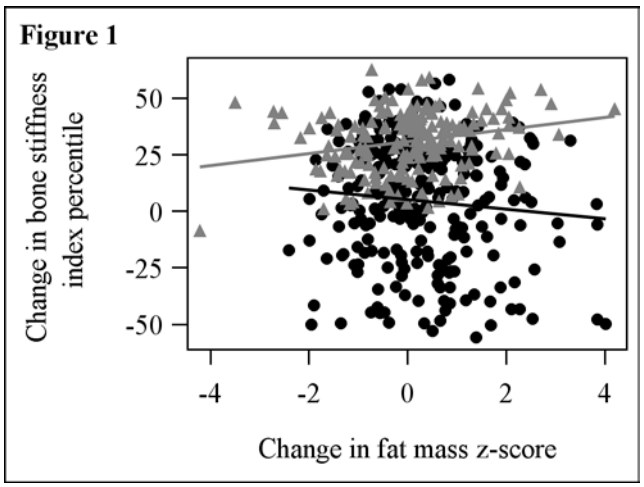

Figure 1 Interaction between change in fat mass z-score and menarche on bone stiffness index percentiles in girls. Accordingly, separate models were stratified by menarche, adjusted for age, socioeconomic status, daylight and bone stiffness index percentiles, height z-score at baseline and change in height z-score, country as a random effect. The black dots and regression line refer to before menarche (β= -1.81, 99%CI: -5.91, 2.30, p= 0.254), whereas the grey triangles and regression line refer to after menarche (β= 3.46, 99%CI: -1.55, 8.47, p= 0.074).

4 Discussion

Overall, we investigated the 2 years and 6 years longitudinal associations between zFM, zFFM and SI percentiles in 2 to 15 years old children and adolescents. Our findings added to the existing evidences that FFM was a significant determinant of bone stiffness development in both sexes. Specifically, baseline zFFM was identified as a positive predictor of change in SI percentiles during 2 years and 6 years follow-up periods. Furthermore, change in zFFM was positively associated with change in SI percentiles after 6 years. These associations were more pronounced in boys compared to girls, and were independent of PA and SB level. In contrast, detrimental effect on bone stiffness accrual may occur with long-term FM increase in boys, whereas the association between the change in FM and bone stiffness in girls depends on maturational status, suggesting a negative association before menarche while a positive association after menarche.

The positive relationship between FFM and bone strength during growth has been well described, most of these bone-related indicators were measured by DXA or pQCT: A number of cross-sectional studies described positive associations between lean mass and weight-bearing bone mass, geometry and architecture in male and female children and adolescents [12, 18, 46]. Few longitudinal studies also suggested that lean mass was a significant predictor of bone strength, and change in lean mass was positively related to change in bone strength [47-49]. Apart from previous findings from the IDEFICS study, there is only a few cross-sectional studies reported the correlation between body composition and calcaneus QUS parameters. For example, in a population of Spanish school children aged 4 to 16 years, FFM were observed positively related to BUA in the calcaneus [50]. In another sample of Malaysian adolescents aged 15-17 years, lean mass was reported to be positively associated with calcaneus BUA [15]. The present study allows an extension to the relatively few longitudinal studies, and adds to the weak evidence that QUS measurements are meaningful for bone development in children and adolescents.

Exploring independent effects of FFM and FM on bone strength are important. There is a consensus that the stimulatory effect exerted by body weight is mainly explained by FFM rather than FM. Findings from several cross-sectional studies supported this conclusion. For example, FM has been shown to be positively correlated to bone strength, while negative associations were observed when lean mass or body weight was included [8, 9, 17, 18]. One study investigated the role of FM and BF% simultaneously and observed an opposite direction of these two parameters in bivariate correlation of cortical bone parameters at the tibia and radius [8]. In a longitudinal pathway analysis, they found the positive association between BMI at 11 years old and whole body bone mineral content (BMC) and bone mineral density (BMD) at age 18 years old was largely mediated by FFM but not FM at age 18 in both female and male adolescents [51]. Our results were consistent with these studies, suggesting a robust and independent effect of FFM on bone stiffness, whereas the potentially predicting effect of FM was attenuated and even reversed after taking FFM into account.

Previous studies have shown that sex differences in body composition and bone are emerging during puberty [52]. On the one hand, we observed the estimate effect of FFM on SI was higher in boys compared to girls after 2 years, and this discrepancy was even more pronounced at 6 years follow-up, which about 40% of participants were considered as in maturity. Findings from a cross-sectional study among 10 to 17 years old healthy children also suggested that the contribution of lean mass to BMC variance was 6–12% in boys, which was larger than 4–10% in girls [53]. These sex differences may partially be explained by the greater FFM and bone size in boys than in girls [54], which may lead to a stronger impact of FFM on bone growth in boys. One the other hand, existing evidences reached contradictory conclusions in the relationship between fat and bone strength across sex groups. For example, Kim et al. [55] found FM was negatively related to total-body-less-head BMD in boys, but was positively associated with BMD of the lumbar spine and femur neck in girls (12 to 19 years old). On the contrary, Zulfarina et al. [15] found FM was negatively associated with SI in 15-17 years old female adolescents rather than male. Further, Sayers et al. [56] found positive

associations between FM and BMC in cortical bone geometry, while these associations were considerably stronger in girls compared to boys, whom were defined as pubertal adolescents in Tanner stages 4 or 5. For now, there is still no consensus in the association between FM and bone strength in male and female during growth, future work should continue to explore the potential mechanisms in sex differences to enhance our knowledge.

Several mechanisms could explain unfavourable changes in bone stiffness after long-term FM increase in boys in our results. Adipose tissue may regulate bone metabolism through exerting adipokines [57], and evidences suggested that adiponectin was inversely related to BMD in childhood and adolescence [58]. Meanwhile, leptin may stimulate osteoblast activity and inhibit osteoclast activity, resulting in increased bone formation and decreased bone resorption [59]. Moreover, adiposity was associated with inflammatory cytokines, and C-reactive protein (CRP) has been related to BMD in healthy adults [60]. However, we didn't find associations or modified effects of CRP in our subsample. Further studies are still needed to clarify the impact of various biological functions of adiposity on bone strength accrual.

Even though we didn't observe any association between FM and bone stiffness in girls, an interaction between menarche and FM gain were observed from our 6 years follow-up data. These results were similar with longitudinal findings from Wey et al. [49], who also found an interaction of FM gain with menarche in females, with the negative associations between FM and total BMC and BMD only existing before menarche. Clark et al. [61] also reported the altered effects of baseline FM on 2 years gain in bone mass and size across different pubertal status, suggested a positive association at Tanner stage 1, no association at stage 2, and a negative association at stage 3 in girls. Hence, it cannot be assumed that relationship between fat and bone strength remains constant over the pubertal status in females. A possible explanation may be attributed to the influence of the rising sexual hormones such as estrogen on bone mass acquisition during puberty, thereby modify the effect of FM on bone metabolism.

Some limitations must be acknowledged in the present study. The major weakness of the study was that body composition was not measured using DXA, which was not feasible in such large-scale cohort among children and adolescents. Instead, skinfold thickness as the best alternatives was used in the present study. The Tanita scale was also used in our study to measure leg-to-leg bioelectrical impedance (ohm) and the Tyrrell formula was used to calculate the FFM (kg) and BF (%) [62]. In order to further clarify our findings, we performed sensitivity analyses with FFM and BF, and found similar results regardless of the technique used. Besides, we could not consider potential confounders such as calcium intake or vitamin D status. Instead, a proxy variable of consumption frequency of milk and dairy products was used for further adjustment, which did not influence the results. Hence we didn't consider this variable in our final analysis in order to not reduce the sample size. Furthermore, no information on maturity at 2 years follow-up was available, and the information at 6 years follow-up was measured by voice change for boys and first menstrual period for girls rather than the Tanner stages. Therefore the interaction effect between body composition and puberty on bone stiffness cannot be further evaluated in the present study. Finally, it is worth to mention the limitation of prospective cohort studies with decreasing sample size over a long follow-up period. In the present study, only 33.4% of the initial baseline cohort with QUS measurements provided follow-up data and complete co-variable information. The differences on some demographic characteristics may cause a possible selective bias. However, our results suggested robust associations of baseline as well as change in body composition with SI percentiles, and these longitudinal associations showed to be stable over 2 and 6 years periods.

5 Conclusions

Our findings highlight the importance of FFM for optimizing bone stiffness during growth. Furthermore, deleterious effect on bone stiffness may occur after relatively long-term exposures to FM gain in boys, while the effect of FM on bone stiffness seems to be opposing across menarche in girls. Future bone health intervention program in children and adolescents should focus on promoting

body composition instead of weight status, particularly differences of sex and maturity also should be taken into consideration.

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Declarations of interest

None

Author contributions

The authors roles are as follow: L.C., W.A. and A.H. conceptualized and designed the study. L.C. conducted the initial analysis and wrote the original draft. H.P. and A.H. assisted with data analysis and interpretation. P.R., T.V., C.C., D.M., G.E., S.D.H., L.M., A.P. contributed to coordination and data collection. All authors revised and improved the manuscript, and approved the final manuscript.

References

[1] H. Johansson, J.A. Kanis, A. Oden, E. McCloskey, R.D. Chapurlat, C. Christiansen, S.R. Cummings, A. Diez-Perez, J.A. Eisman, S. Fujiwara, C.C. Gluer, D. Goltzman, D. Hans, K.T. Khaw, M.A. Krieg, H. Kroger, A.Z. LaCroix, E. Lau, W.D. Leslie, D. Mellstrom, L.J. Melton, 3rd, T.W. O'Neill, J.A. Pasco, J.C. Prior, D.M. Reid, F. Rivadeneira, T. van Staa, N. Yoshimura, M.C. Zillikens, A meta-analysis of the association of fracture risk and body mass index in women, Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research 29(1) (2014) 223-33.

[2] S. Yang, L.M. Lix, L. Yan, A.M. Hinds, W.D. Leslie, International Classification of Diseases (ICD)-coded obesity predicts risk of incident osteoporotic fracture, PloS one 12(12) (2017) e0189168.

[3] J.N. Farr, P. Dimitri, The Impact of Fat and Obesity on Bone Microarchitecture and Strength in Children, Calcif Tissue Int 100(5) (2017) 500-513.

[4] I.R. Reid, Relationships between fat and bone, Osteoporos Int 19(5) (2008) 595-606.

[5] B.L. Specker, N. Johannsen, T. Binkley, K. Finn, Total body bone mineral content and tibial cortical bone measures in preschool children, Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research 16(12) (2001) 2298-305.

[6] R.G. Sudhagoni, H.E. Wey, G.D. Djira, B.L. Specker, Longitudinal effects of fat and lean mass on bone accrual in infants, Bone 50(3) (2012) 638-42.

[7] K.S. Wosje, P.R. Khoury, R.P. Claytor, K.A. Copeland, H.J. Kalkwarf, S.R. Daniels, Adiposity and TV viewing are related to less bone accrual in young children, The Journal of pediatrics 154(1) (2009) 79-85 e2.

[8] N.K. Pollock, E.M. Laing, C.A. Baile, M.W. Hamrick, D.B. Hall, R.D. Lewis, Is adiposity advantageous for bone strength? A peripheral quantitative computed tomography study in late adolescent females, Am J Clin Nutr 86(5) (2007) 1530-8.

[9] J.N. Farr, Z. Chen, J.R. Lisse, T.G. Lohman, S.B. Going, Relationship of total body fat mass to weight-bearing bone volumetric density, geometry, and strength in young girls, Bone 46(4) (2010) 977-84.

[10] D.R. Laddu, J.N. Farr, M.J. Laudermilk, V.R. Lee, R.M. Blew, C. Stump, L. Houtkooper, T.G. Lohman, S.B. Going, Longitudinal relationships between whole body and central adiposity on weight-bearing bone geometry, density, and bone strength: a pQCT study in young girls, Arch Osteoporos 8 (2013) 156.

[11] K.B. Dorsey, J.C. Thornton, S.B. Heymsfield, D. Gallagher, Greater lean tissue and skeletal muscle mass are associated with higher bone mineral content in children, Nutr Metab (Lond) 7 (2010) 41.

[12] B. Guo, Q. Wu, J. Gong, Z. Xiao, Y. Tang, J. Shang, Y. Cheng, H. Xu, Relationships between the lean mass index and bone mass and reference values of muscular status in healthy Chinese children and adolescents, Journal of bone and mineral metabolism 34(6) (2016) 703-713.

[13] M. Locquet, C. Beaudart, N. Durieux, J.Y. Reginster, O. Bruyere, Relationship between the changes over time of bone mass and muscle health in children and adults: a systematic review and meta-analysis, BMC Musculoskelet Disord 20(1) (2019) 429.

[14] D. Herrmann, C. Buck, I. Sioen, Y. Kouride, S. Marild, D. Molnar, T. Mouratidou, Y. Pitsiladis, P. Russo, T. Veidebaum, W. Ahrens, I. consortium, Impact of physical activity, sedentary behaviour and muscle strength on bone stiffness in 2-10-year-old children-cross-sectional results from the IDEFICS study, Int J Behav Nutr Phys Act 12 (2015) 112.

[15] M.S. Zulfarina, R. Sharif, S.B. Syarifah-Noratiqah, A.M. Sharkawi, Z.S. Aqilah-Sm, S.A. Mokhtar, S.A. Nazrun, I. Naina-Mohamed, M.r. group, Modifiable factors associated with bone health in Malaysian adolescents utilising calcaneus quantitative ultrasound, PloS one 13(8) (2018) e0202321.

[16] R.P. El Hage, D. Courteix, C.L. Benhamou, C. Jacob, C. Jaffre, Relative importance of lean and fat mass on bone mineral density in a group of adolescent girls and boys, Eur J Appl Physiol 105(5) (2009) 759-64.

[17] L. Gracia-Marco, F.B. Ortega, D. Jimenez-Pavon, G. Rodriguez, M.J. Castillo, G. Vicente-Rodriguez, L.A. Moreno, Adiposity and bone health in Spanish adolescents. The HELENA study, Osteoporos Int 23(3) (2012) 937-47.

[18] A. Janicka, T.A. Wren, M.M. Sanchez, F. Dorey, P.S. Kim, S.D. Mittelman, V. Gilsanz, Fat mass is not beneficial to bone in adolescents and young adults, J Clin Endocrinol Metab 92(1) (2007) 143-7.

[19] I. Sioen, T. Mouratidou, D. Herrmann, S. De Henauw, J.M. Kaufman, D. Molnar, L.A. Moreno, S. Marild, G. Barba, A. Siani, F. Gianfagna, M. Tornaritis, T. Veidebaum, W. Ahrens, Relationship between markers of body fat and calcaneal bone stiffness differs between preschool and primary school children: results from the IDEFICS baseline survey, Calcif Tissue Int 91(4) (2012) 276-85.

[20] G.I. Baroncelli, Quantitative ultrasound methods to assess bone mineral status in children: technical characteristics, performance, and clinical application, Pediatr Res 63(3) (2008) 220-8.

[21] M.A. Krieg, R. Barkmann, S. Gonnelli, A. Stewart, D.C. Bauer, L. Del Rio Barquero, J.J. Kaufman, R. Lorenc, P.D. Miller, W.P. Olszynski, C. Poiana, A.M. Schott, E.M. Lewiecki, D. Hans, Quantitative ultrasound in the management of osteoporosis: the 2007 ISCD Official Positions, J Clin Densitom 11(1) (2008) 163-87.

[22] P. Trimpou, I. Bosaeus, B.A. Bengtsson, K. Landin-Wilhelmsen, High correlation between quantitative ultrasound and DXA during 7 years of follow-up, Eur J Radiol 73(2) (2010) 360-4.

[23] K.T. Khaw, J. Reeve, R. Luben, S. Bingham, A. Welch, N. Wareham, S. Oakes, N. Day, Prediction of total and hip fracture risk in men and women by quantitative ultrasound of the calcaneus: EPIC-Norfolk prospective population study, Lancet 363(9404) (2004) 197-202.

[24] M. Jaworski, M. Lebiedowski, R.S. Lorenc, J. Trempe, Ultrasound bone measurement in pediatric subjects, Calcif Tissue Int 56(5) (1995) 368-71.

[25] M. Sundberg, P. Gardsell, O. Johnell, E. Ornstein, I. Sernbo, Comparison of quantitative ultrasound measurements in calcaneus with DXA and SXA at other skeletal sites: a population-based study on 280 children aged 11-16 years, Osteoporos Int 8(5) (1998) 410-7.

[26] Y. Xu, B. Guo, J. Gong, H. Xu, Z. Bai, The correlation between calcaneus stiffness index calculated by QUS and total body BMD assessed by DXA in Chinese children and adolescents, Journal of bone and mineral metabolism 32(2) (2014) 159-66.

[27] W. Ahrens, K. Bammann, A. Siani, K. Buchecker, S. De Henauw, L. Iacoviello, A. Hebestreit, V. Krogh, L. Lissner, S. Marild, D. Molnar, L.A. Moreno, Y.P. Pitsiladis, L. Reisch, M. Tornaritis, T. Veidebaum, I. Pigeot, I. Consortium, The IDEFICS cohort: design, characteristics and participation in the baseline survey, Int J Obes (Lond) 35 Suppl 1 (2011) S3-15.

[28] W. Ahrens, A. Siani, R. Adan, S. De Henauw, G. Eiben, W. Gwozdz, A. Hebestreit, M. Hunsberger, J. Kaprio, V. Krogh, L. Lissner, D. Molnar, L.A. Moreno, A. Page, C. Pico, L. Reisch, R.M. Smith, M. Tornaritis, T. Veidebaum, G. Williams, H. Pohlabeln, I. Pigeot, I.F. consortium, Cohort Profile: The transition from childhood to adolescence in European children-how I.Family extends the IDEFICS cohort, Int J Epidemiol 46(5) (2017) 1394-1395j.

[29] C.F. Njeh, C.M. Boivin, C.M. Langton, The role of ultrasound in the assessment of osteoporosis: a review, Osteoporos Int 7(1) (1997) 7-22.

[30] D. Herrmann, T. Intemann, F. Lauria, S. Marild, D. Molnar, L.A. Moreno, I. Sioen, M. Tornaritis, T. Veidebaum, I. Pigeot, W. Ahrens, I. consortium, Reference values of bone stiffness index and C-terminal telopeptide in healthy European children, Int J Obes (Lond) 38 Suppl 2 (2014) S76-85.

[31] G. Rodriguez, L.A. Moreno, M.G. Blay, V.A. Blay, J. Fleta, A. Sarria, M. Bueno, A.V.-Z.S. Group, Body fat measurement in adolescents: comparison of skinfold thickness equations with dual-energy X-ray absorptiometry, Eur J Clin Nutr 59(10) (2005) 1158-66.

[32] M.H. Slaughter, T.G. Lohman, R.A. Boileau, C.A. Horswill, R.J. Stillman, M.D. Van Loan, D.A. Bemben, Skinfold equations for estimation of body fatness in children and youth, Hum Biol 60(5) (1988) 709-23.

[33] S. Stomfai, W. Ahrens, K. Bammann, E. Kovacs, S. Marild, N. Michels, L.A. Moreno, H. Pohlabeln, A. Siani, M. Tornaritis, T. Veidebaum, D. Molnar, I. Consortium, Intra- and inter-observer reliability in anthropometric measurements in children, Int J Obes (Lond) 35 Suppl 1 (2011) S45-51.

[34] M. Suling, A. Hebestreit, J. Peplies, K. Bammann, A. Nappo, G. Eiben, J.M. Alvira, V. Verbestel, E. Kovacs, Y.P. Pitsiladis, T. Veidebaum, C. Hadjigeorgiou, K. Knof, W. Ahrens, I. Consortium, Design and results of the pretest of the IDEFICS study, Int J Obes (Lond) 35 Suppl 1 (2011) S30-44.

[35] P. Nagy, E. Kovacs, L.A. Moreno, T. Veidebaum, M. Tornaritis, Y. Kourides, A. Siani, F. Lauria, I. Sioen, M. Claessens, S. Marild, L. Lissner, K. Bammann, T. Intemann, C. Buck, I. Pigeot, W. Ahrens, D. Molnar, I. consortium, Percentile reference values for anthropometric body composition indices in European children from the IDEFICS study, Int J Obes (Lond) 38 Suppl 2 (2014) S15-25.

[36] K. Bammann, I. Huybrechts, G. Vicente-Rodriguez, C. Easton, T. De Vriendt, S. Marild, M.I. Mesana, M.W. Peeters, J.J. Reilly, I. Sioen, B. Tubic, N. Wawro, J.C. Wells, K. Westerterp, Y. Pitsiladis, L.A. Moreno, I. Consortium, Validation of anthropometry and foot-to-foot bioelectrical resistance against a three-component model to assess total body fat in children: the IDEFICS study, Int J Obes (Lond) 37(4) (2013) 520-6.

[37] K. Bammann, I. Sioen, I. Huybrechts, J.A. Casajus, G. Vicente-Rodriguez, R. Cuthill, K. Konstabel, B. Tubic, N. Wawro, M. Rayson, K. Westerterp, S. Marild, Y.P. Pitsiladis, J.J. Reilly, L.A. Moreno, S. De Henauw, I. Consortium, The IDEFICS validation study on field methods for assessing physical activity and body composition in children: design and data collection, Int J Obes (Lond) 35 Suppl 1 (2011) S79-87.

[38] U. Hagg, J. Taranger, Menarche and voice change as indicators of the pubertal growth spurt, Acta Odontol Scand 38(3) (1980) 179-86.

[39] A. Juul, S. Magnusdottir, T. Scheike, S. Prytz, N.E. Skakkebaek, Age at voice break in Danish boys: effects of pre-pubertal body mass index and secular trend, Int J Androl 30(6) (2007) 537-42.

[40] A. Juul, G. Teilmann, T. Scheike, N.T. Hertel, K. Holm, E.M. Laursen, K.M. Main, N.E. Skakkebaek, Pubertal development in Danish children: comparison of recent European and US data, Int J Androl 29(1) (2006) 247-55; discussion 286-90.

[41] D. Charalampopoulos, A. McLoughlin, C.E. Elks, K.K. Ong, Age at menarche and risks of all-cause and cardiovascular death: a systematic review and meta-analysis, Am J Epidemiol 180(1) (2014) 29-40.

[42] K.K. Ong, D. Bann, A.K. Wills, K. Ward, J.E. Adams, R. Hardy, D. Kuh, H. National Survey of, S. Development, T. Data Collection, Timing of voice breaking in males associated with growth and weight gain across the life course, J Clin Endocrinol Metab 97(8) (2012) 2844-52.

[43] UNESCO, International Standard Classification of Education, 2010. http://www.uis.unesco.org/Education/Pages/international-standardclassification-of-education.aspx.

[44] T.J. Cole, J.V. Freeman, M.A. Preece, British 1990 growth reference centiles for weight, height, body mass index and head circumference fitted by maximum penalized likelihood, Stat Med 17(4) (1998) 407-29.

[45] A. Cranney, T. Horsley, S. O'Donnell, H. Weiler, L. Puil, D. Ooi, S. Atkinson, L. Ward, D. Moher, D. Hanley, M. Fang, F. Yazdi, C. Garritty, M. Sampson, N. Barrowman, A. Tsertsvadze, V.

Mamaladze, Effectiveness and safety of vitamin D in relation to bone health, Evid Rep Technol Assess (Full Rep) (158) (2007) 1-235.

[46] M. Jeddi, M.H. Dabbaghmanesh, G. Ranjbar Omrani, S.M. Ayatollahi, Z. Bagheri, M. Bakhshayeshkaram, Relative Importance of Lean and Fat Mass on Bone Mineral Density in Iranian Children and Adolescents, Int J Endocrinol Metab 13(3) (2015) e25542.

[47] S. Dalskov, C. Ritz, A. Larnkjaer, C.T. Damsgaard, R.A. Petersen, L.B. Sorensen, K.K. Ong, A. Astrup, K.F. Michaelsen, C. Molgaard, Associations between adiposity, hormones, and gains in height, whole-body height-adjusted bone size, and size-adjusted bone mineral content in 8- to 11-year-old children, Osteoporos Int 27(4) (2016) 1619-1629.

[48] R.J. Wetzsteon, M.A. Petit, H.M. Macdonald, J.M. Hughes, T.J. Beck, H.A. McKay, Bone structure and volumetric BMD in overweight children: a longitudinal study, Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research 23(12) (2008) 1946-53.

[49] H.E. Wey, T.L. Binkley, T.M. Beare, C.L. Wey, B.L. Specker, Cross-sectional versus longitudinal associations of lean and fat mass with pQCT bone outcomes in children, J Clin Endocrinol Metab 96(1) (2011) 106-14.

[50] J.M. Lavado-Garcia, J.F. Calderon-Garcia, J.M. Moran, M.L. Canal-Macias, T. Rodriguez-Dominguez, J.D. Pedrera-Zamorano, Bone mass of Spanish school children: impact of anthropometric, dietary and body composition factors, Journal of bone and mineral metabolism 30(2) (2012) 193-201.

[51] L.C. Muniz, A.M. Menezes, M.C. Assuncao, J. Martinez-Mesa, F.C. Wehrmeister, L.D. Howe, P.C. Hallal, H. Goncalves, F.C. Barros, Body mass index at 11 years and bone mass at age 18: path analysis within the 1993 Pelotas (Brazil) birth cohort study, BMC Musculoskelet Disord 16 (2015) 71.

[52] E. Schoenau, C.M. Neu, E. Mokov, G. Wassmer, F. Manz, Influence of puberty on muscle area and cortical bone area of the forearm in boys and girls, J Clin Endocrinol Metab 85(3) (2000) 1095-8.

[53] A. Arabi, H. Tamim, M. Nabulsi, J. Maalouf, H. Khalife, M. Choucair, R. Vieth, G. El-Hajj Fuleihan, Sex differences in the effect of body-composition variables on bone mass in healthy children and adolescents, Am J Clin Nutr 80(5) (2004) 1428-35.

[54] J.N. Farr, S. Amin, N.K. LeBrasseur, E.J. Atkinson, S.J. Achenbach, L.K. McCready, L. Joseph Melton, 3rd, S. Khosla, Body composition during childhood and adolescence: relations to bone strength and microstructure, J Clin Endocrinol Metab 99(12) (2014) 4641-8.

[55] H.Y. Kim, H.W. Jung, H. Hong, J.H. Kim, C.H. Shin, S.W. Yang, Y.A. Lee, The Role of Overweight and Obesity on Bone Health in Korean Adolescents with a Focus on Lean and Fat Mass, J Korean Med Sci 32(10) (2017) 1633-1641.

[56] A. Sayers, J.H. Tobias, Fat mass exerts a greater effect on cortical bone mass in girls than boys, J Clin Endocrinol Metab 95(2) (2010) 699-706.

[57] E. Biver, C. Salliot, C. Combescure, L. Gossec, P. Hardouin, I. Legroux-Gerot, B. Cortet, Influence of adipokines and ghrelin on bone mineral density and fracture risk: a systematic review and meta-analysis, J Clin Endocrinol Metab 96(9) (2011) 2703-13.

[58] A. Sayers, N.J. Timpson, N. Sattar, J. Deanfield, A.D. Hingorani, G. Davey-Smith, J.H. Tobias, Adiponectin and its association with bone mass accrual in childhood, Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research 25(10) (2010) 2212-20.

[59] V. Cirmanova, M. Bayer, L. Starka, K. Zajickova, The effect of leptin on bone: an evolving concept of action, Physiol Res 57 Suppl 1 (2008) S143-51.

[60] H.S. Lim, Y.H. Park, S.K. Kim, Relationship between Serum Inflammatory Marker and Bone Mineral Density in Healthy Adults, J Bone Metab 23(1) (2016) 27-33.

[61] E.M. Clark, A.R. Ness, J.H. Tobias, Adipose tissue stimulates bone growth in prepubertal children, J Clin Endocrinol Metab 91(7) (2006) 2534-41.

[62] V.J. Tyrrell, G. Richards, P. Hofman, G.F. Gillies, E. Robinson, W.S. Cutfield, Foot-to-foot bioelectrical impedance analysis: a valuable tool for the measurement of body composition in children, Int J Obes Relat Metab Disord 25(2) (2001) 273-8.

Paper 2

Cross-sectional and longitudinal associations between physical activity, sedentary behavior and bone stiffness index across weight status in European children and adolescents

Lan Cheng, Hermann Pohlabeln, Wolfgang Ahrens, Fabio Lauria, Toomas Veidebaum, Charalambos Chadjigeorgiou, Dénes Molnár, Gabriele Eiben, Nathalie Michels, Luis A. Moreno, Angie S. Page, Yannis Pitsiladis, Antje Hebestreit, On behalf of the IDEFICS and I.Family Consortia

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Cross-sectional and longitudinal associations between physical activity, sedentary behaviour and bone stiffness index across weight status in European children and adolescents

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Abstract

Background: The associations between physical activity (PA), sedentary behaviour (SB) and bone health may be differentially affected by weight status during growth. This study aims to assess the cross-sectional and longitudinal associations between PA, SB and bone stiffness index (SI) in European children and adolescents, taking the weight status into consideration.

Methods: Calcaneus SI was first measured by quantitative ultrasound among children aged 2–9 years old in 2007/08. It was measured again after 2 years in the IDEFICS study and after 6 years in the I. Family study. A sample of 2008 participants with time spent at sports clubs, watching TV and playing computer/games self-reported by questionnaire, and a subsample of 1037 participants with SB, light PA (LPA) and moderate-to-vigorous PA (MVPA) objectively measured using Actigraph accelerometers were included in the analyses. Weight status was defined as thin/normal and overweight/obese according to the extended International Obesity Task Force criteria. Linear mixed-effects models were used to estimate the cross-sectional and longitudinal associations between PA, SB and SI percentiles, stratified by weight status.

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(Continued from previous page)
Results: The cross-sectional association between weekly duration of watching TV and SI percentiles was negative in thin/normal weight group (β = − 0.35, $p = 0.008$). However, baseline weekly duration of watching TV (β = − 0.63, $p = 0.021$) and change after 2 years (β = − 0.63, $p = 0.022$) as well as the change in weekly duration of playing computer/games after 6 years (β = − 0.75, $p = 0.019$) were inversely associated with corresponding changes in SI percentiles in overweight/obese group. Change in time spent at sports clubs was positively associated with change in SI percentiles after 2 years (β = 1.28, $p = 0.001$), with comparable effect sizes across weight status. In the subsample with accelerometer data, we found a positive cross-sectional association between MVPA and SI percentiles in thin/normal weight group. Baseline MVPA predicted changes in SI percentiles after 2 and 6 years in all groups.

Conclusions: Our results suggested the beneficial effect of PA on SI. However, the increasing durations of screen-based SB might be risk factors for SI development, especially in overweight/obese children and adolescents.

Keywords: Physical activity, Sedentary behaviour, Overweight, Bone stiffness index, Observational study

Background

Bone strength is influenced by mass, architecture and density, while the trajectory of bone strength accrual persists up to the age of about 18 years until peak bone mass (PBM) is reached [1, 2]. Even though PBM is mainly explained by genetic determinants [3], it is also influenced by lifestyle-related factors such as mechanical loading, physical activity (PA), sedentary behaviour (SB) and nutrition [4, 5]. Further, PBM is an important predictor of osteoporosis in adults, due to the age-related bone loss that occurs over time [6]. Hence, in order to prevent fractures and osteoporosis in later life, it is important to initiate preventive measures during childhood and adolescence.

The positive osteogenic effect of PA, in particular weight-bearing exercises (WBEs), on bone strength seems to be irrefutable [7, 8]. However, despite these proven health benefits, the secular trend of PA shows a decrease among European children and adolescents, with most of them not meeting the World Health Organization (WHO) recommendations for PA [9–11]. Together with the decrease of PA, high levels of SB among this population group now constitute a serious public health concern [12]. In recent studies, the total duration of SB among 10- to 12-year-old European children was reported to be nearly 8 h per day [13], and they were also observed to spend more than 2 h per day in front of computer or TV screens [14]. The debate on the detrimental effects of SB on bone strength is, however, more controversial compared to beneficial effects of PA. Previous studies reported a negative [15] or null [16] association between the total duration of objectively measured SB using accelerometers and bone strength, while others suggested that self-reported screen-based SB such as using the internet [17], watching TV [18] and total screen time [19] may inversely influence bone mass. Currently, more studies are needed to combine self-reported data with objectively measured data when examining the short- and long-term effects of context-specific PA and SB on bone strength in young populations.

On one hand, sedentary lifestyles may be associated with poor bone health and are also linked to a higher risk of overweight and obesity in children and adolescents [20]. On the other hand, previous studies indicated that overweight or obese children have higher bone mass [21] or strength [22] compared to their normal weight peers, which, however, is in conflict with the unfavourable effects of sedentary lifestyles. In addition, being overweight has been reported to increase the risk for sedentary lifestyles [23], thereby leading to poor bone health. In view of these potential pathways, the associations between PA, SB and bone strength may be differentially influenced by overweight and obesity.

Understanding which specific dimensions of PA and SB influence the growing skeleton is crucial for the development of effective and sustainable strategies for increased bone strength. Particularly the role of weight status in these associations is still poorly understood. In an effort to fill this gap, information on the bone stiffness index (SI) measured using quantitative ultrasound (QUS) as a proxy indicator for bone strength has been repeatedly collected in a sample from the IDEFICS (Identification and prevention of dietary- and lifestyle-induced health effects in children and infants) and I. Family studies. Self-reported time spent at sports club, WBEs, watching TV and playing computer/games, as well as objectively measured SB, light PA (LPA) and moderate-to-vigorous PA (MVPA) were also collected to assess the cross-sectional and longitudinal associations of various kinds of PA and SB on SI in European children and adolescents across different weight statuses.

Methods

Study sample

The IDEFICS/I.Family study is the largest prospective child cohort in Europe with repeated measurements of

anthropometric indicators, clinical examinations as well as extensive questionnaire-based information on socio-demographic factors, PA and nutrition [24, 25]. The first two waves of data collection occurred in the context of the IDEFICS study, which comprised 16,229 children from eight European countries (Belgium, Cyprus, Estonia, Germany, Hungary, Italy, Spain and Sweden), who were aged 2–9.9 years at baseline between September 2007 and May 2008. The second wave between September 2009 and May 2010 included a follow-up of 11,043 children from baseline and 2543 newly recruited children. The third wave was conducted in the context of the I. Family study between January 2013 and June 2014 with a follow-up of 7117 children from the original IDEFICS cohort and 2501 newly recruited children. All parents provided signed informed consent for their children prior to all exanimations. In addition, children younger than 12 years gave their oral consent and children above 12 years provided a signed simplified form of consent. Ethical approval for the study was obtained from the ethics committees for participating centres in each country.

Inclusion and exclusion criteria

QUS measurements were obtained as an optional module in a subgroup of participants in the IDEFICS study, while in the I. Family study, QUS data were only available for five of the eight participating countries. We assumed that no substantial selection effects occurred since the reduced participation was mainly because of budgetary constraints and device feasibility. In order to simultaneously investigate cross-sectional and longitudinal associations between the exposures of interest and bone SI, we included 3422 children with baseline and at least one follow-up QUS measurements of both the left and right foot. In accordance with findings from a previous IDEFICS study on QUS measurement precision, there was a significant discrepancy in SI difference between the left and right foot across devices in each participating centre (unpublished data). In order to control for this discrepancy, 200 children whose QUS measurements had an SI difference between the left and right foot above 41 units (97th percentile, calculated based on 7612 repeated measurements in total) were excluded. A further 43 children who at baseline reported having medical conditions preventing participation in regular PA and/or known to influence bone metabolism were excluded [26].

Finally, children without self-reported PA, screen-based SB or covariate data were excluded, leaving a total of 2008 participants for the full analysis. The mean age of the main sample was 6.14 years (SD = 1.80), 54.1% were boys and the proportions of low, medium and high familial socio-economic status (SES) were 10.4, 56.3 and 33.3%, respectively. A subsample of 1037 participants who provided objectively measured accelerometer-based SB, LPA and MVPA data was also analysed. The mean age of the subsample was 6.45 years (SD = 1.72), 50.4% were boys and the proportions of low, medium and high familial socio-economic status (SES) were 9.4, 60.3 and 30.3%, respectively. Compared with the original IDEFICS study sample, the children in both analytic samples were older (vs. 6.01 years, SD = 1.79) and more children had low (vs. 9.0%) and medium levels (vs. 50.3%) of SES. In addition, more boys were included in the main sample (vs. 50.8%). The inclusion and exclusion process of participants for the final analysis is summarised in Fig. 1. No children from Cyprus were included in the analysis as they did not fulfil any of the inclusion criteria.

Bone stiffness index

QUS measurements on the left and right calcaneus were performed using Achilles Lunar Insight™ (GE Healthcare, Milwaukee, WI, USA). The parameters of speed of sound (SOS, m/s) and broadband ultrasound attenuation (BUA, dB/MHz) assessed by QUS devices reflect the velocity and attenuation of the ultrasound waves through the bone tissue, respectively. The SI value was estimated automatically by Lunar Achilles OsteoReport Software and reported as 'unit' according to the equation: SI = (0.67*BUA) + (0.28*SOS) − 420, with high SI values indicating better bone strength [27]. SOS, BUA and SI assessed by calcaneus QUS have been shown to be correlated with bone mineral content (BMC) and bone mineral density (BMD) assessed by dual energy X-ray absorptiometry (DXA) in children and adolescents in previous studies [28, 29]. There is also evidence suggesting that QUS devices could be used to estimate fracture risk and osteoporosis in childhood [27] and adulthood [30]. Compared to DXA, the main advantages of QUS devices are that they are non-radiating, quick and cost-effective, making them more suitable for large-scale epidemiological studies, particularly in healthy young populations. The SI value not only reflects bone density, it is also influenced by the architecture and elasticity of the bone tissue, which makes it possible to provide some structural information [27]. The reproducibility in each QUS device was tested on 91 children from the IDEFICS baseline; no differences were found in SI values between the three repeated measurements and between measurements at the left and right foot. The root-mean-square coefficients of variation (CV_{RMS}) for the SI measurements were 7.2 and 9.2% on the left and right foot, respectively (unpublished data). In line with the study protocol, daily machine calibration was carried out during the entire study period and the measurements were taken by trained nurses according to the standard procedure [31]. Two different sizes of foot adapters were

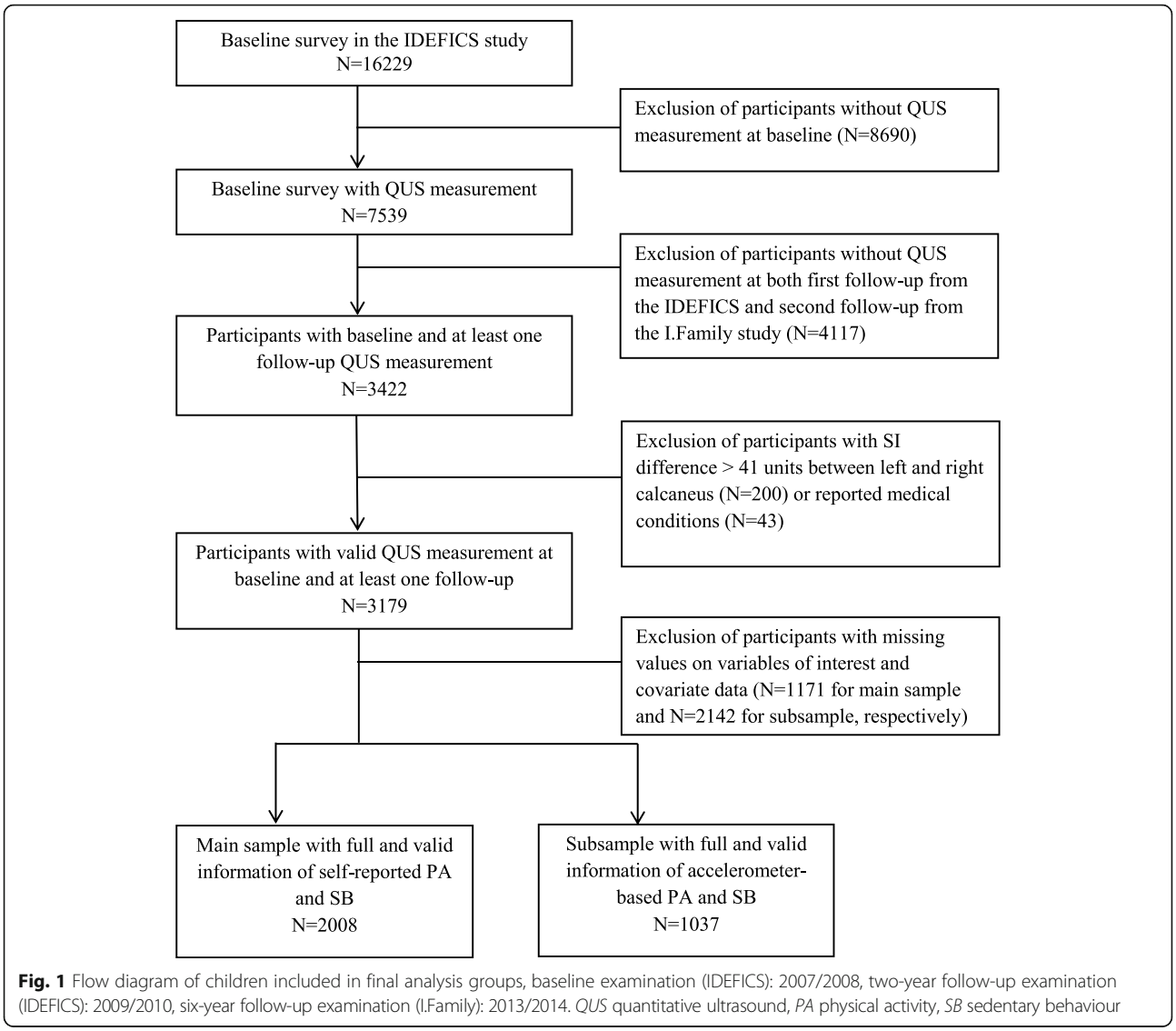

Fig. 1 Flow diagram of children included in final analysis groups, baseline examination (IDEFICS): 2007/2008, two-year follow-up examination (IDEFICS): 2009/2010, six-year follow-up examination (I.Family): 2013/2014. *QUS* quantitative ultrasound, *PA* physical activity, *SB* sedentary behaviour

used to put the calcaneus in an appropriate position. The mean SI of the left and right calcaneus was calculated and used in the statistical analysis. The distribution of SI was assessed and age-, sex- and height-specific percentiles for SI values were calculated as outcomes [32].

Anthropometric measurements

Height and weight were measured in light clothing without shoes. The former was measured to the nearest 0.1 cm using a standard clinical Seca 225 stadiometer (Seca, Hamburg, Germany) and the latter to the nearest 0.1 kg using a BC420 SMA scale (Tanita, Amsterdam, the Netherlands). The intra- and inter-observer reliability for height and weight were conducted in each centre, with CV% ranging from 0.2 to 1.0% in the IDEFICS [33] and I. Family study (unpublished data). In all study centres, trained nurses took the measurements following standardised procedures. For each child, age- and sex-specific z-scores of height and weight were determined using the LMS method by Cole et al. [34]. Body mass index (BMI, kg/m^2) was calculated as body weight divided by squared body height, and weight status (thin/normal and overweight/obese) was classified at the 90th percentile (passing through the BMI of 25 at the age of 18) as recommended based on the extended International Obesity Task Force (IOTF) BMI criteria [35].

Questionnaires

The questionnaires relating to lifestyle behaviours were answered by parents for young children up to 11 years old; they were self-reported for 12- to 15-year-old adolescents. The following information was collected for each child/adolescent: whether they were a member of a sports club and if so, 1) how many hours and minutes

per week they spent there and 2) in what kind of sports they participated at the sports club. The time spent at sports clubs was calculated by adding the hours and minutes reported and expressed as hours per week (h/w). The variable WBE was based on all reported types of sports, classified according to the loads and categorised into moderate or high mechanical loads (ball games, gymnastics, dancing, skating, martial arts and athletics, etc.) and no or low mechanical loads (swimming, biking and horseback riding, etc.). In addition, information regarding the time usually spent watching TV/videos/DVDs and playing on a computer/game console on a normal weekday and weekend day was also collected. For both questions, six response categories were offered and converted into the following scoring system: not at all = 0, < 30 min = 1, < 1 h = 2, 1- < 2 h = 3, 2–3 h = 4, and > 3 h = 5. Each screen-based SB was calculated separately for weekdays and weekend days by adding the converted responses of the individual questions and expressed as hours per week (h/w). Weekly duration of watching TV was further classified into > 14 h/w and ≤ 14 h/w in accordance with international guidelines [36, 37] to investigate the benefit of fulfilling the guidelines on SI.

Accelerometer data

PA and SB were objectively measured using Actigraph accelerometer devices (Actigraph, LLC, Pensacola, FL, USA) in a subsample of participants. Parents or legal guardians were asked to ensure that their child wore the accelerometer on the right hip and that it was only removed during water-based activities and bedtime. Data were collected in the vertical axis for three-axial accelerometer. In the IDEFICS study, either the GT1M or ActiTrainer was used; the sensor units of both models are identical. In previous validation studies, both types of accelerometers have been observed to measure comparable MPA, LPA and MVPA levels [38]. However, the outputs of counts per minute (cpm) [39] and low PA levels (e.g. LPA and walking) [38] for ActiTrainer were lower than other Actigraph models, thus care should be taken when interpreting these results. In the I. Family study, either the GT1M or GT3x + was used, the comparability of the vertical axis outputs for the GT3X and GT1M has also been proven in previous studies [40, 41]. The participants were requested to wear the accelerometer for at least 3 days including one weekend day in the IDEFICS study and for 7 days in the I. Family study. Participants were included in the analyses only if they had at least 6 h of data per day and three accelerometer measurement days. Further, any periods containing 20 min or more of consecutive zero counts were removed as non-wearing time. All accelerometer recordings were integrated over 60s epochs and the intensity levels were classified as SB (≤100 cpm), LPA (> 100- < 2296 cpm) and MVPA (≥2296 cpm) according to the cut-off points suggested by Evenson et al. [42]. More details on processing of accelerometer data in the IDEFICS/I.Family study have been published elsewhere [9, 43]. In the present study, total durations of objectively measured SB and LPA were expressed as hours per day (h/d). In order to better interpret the regression coefficients, the unit of objectively measured MVPA was converted to 10 min per day (10 min/d) according to previous studies, reporting that every additional 10 min/d of MVPA was associated with increases of bone health indicators in children [26, 44]. We further considered the variable objectively measured MVPA as a dichotomised instead of a continuous variable. According to WHO recommended levels of PA for children and adolescents aged 5–17 years old, daily duration of objectively measured MVPA ≥1 h/d was regarded as adhering to the guideline [45].

Confounding variables

Sex, age and questions regarding the familial SES of participants were reported by parents. SES was assessed based on the highest educational level of parents according to the International Standard Classification of Education (ISCED) and categorised into low (ISCED 0,1,2), medium (ISCED 3,4) and high (ISCED 5,6) [46]. The voice change of boys and the first menstrual period of girls from the age of 8 years old were collected in the I. Family study as a proxy for pubertal development and further categorised into pre-pubertal and pubertal in the present study. Both indicators have been widely used to assess maturation in previous epidemiological studies, suggesting that changes in the male voice often occur between Tanner stages 3 and 4 [47, 48], which is the comparable onset age of menarche in females [49]. The variance between countries was also considered. Further, sunlight exposure as the most important source for vitamin D synthesis was also taken into consideration [50], calculated by mean daylight duration for each examination month in each location based on astronomical tables [26].

Statistical analyses

All analyses were performed using SAS software (V9.3; SAS Institute Inc., Cary, North Carolina, USA). The changes in continuous variables were determined by calculating the differences between follow-up after 2 or 6 years and baseline values. Descriptive statistics, e.g. means, standard deviations (SD), and frequencies for baseline and changes of each variable were conducted and stratified by weight status (thin/normal and overweight/obese) in each survey. Differences for continuous variables were compared using t tests, and chi-square tests were used for categorical variables.

Linear mixed-effects models were used to estimate the cross-sectional and longitudinal associations between PA, SB and SI percentiles, with country as a random effect (at the level of the intercept). To avoid getting associations that are irrelevantly statistically significant, for instance simply due to the large sample size or to multiple testing, a more stringent criterion for statistical significance ($\alpha = 0.01$) was chosen. Regression coefficients (β) and 99%-confidence intervals (99%CI) were estimated in all models. The cross-sectional analyses were based on the data from baseline and the outcome was baseline SI percentiles. Weekly duration of watching TV, playing computer/games and sports club activities as well as WBE were taken into consideration as exposures and adjusted for sex, age, SES, daylight duration, weight and height z-scores. In the longitudinal analyses, the outcomes were the changes in SI percentiles after 2 or 6 years, with taking the baseline and changes in exposures into consideration. In addition to the confounding factors described above, we also included baseline SI percentiles and pubertal status in the longitudinal models. Based on the same analytical approach, objectively measured SB, LPA and MVPA were considered in subgroup analyses and presented separately. All analyses were performed in the whole group and then further stratified by thin/normal and overweight/obese groups. Differences in the association between each exposure of interest and corresponding outcome across weight status were further tested by interactive terms in the whole group models, however, they were not considered in the final analyses since no statistically significant interactions were observed.

Results

Descriptive characteristics of study population

As summarised in Table 1, the baseline proportions of overweight/obese children in the main sample ($n = 2008$) and subsample with accelerometer data ($n = 1037$) were 19.0 and 19.7%, respectively. At individual country-level, Italy had the highest proportion of overweight/obese children and Belgium the lowest. Regarding SI, in the main sample, the means of SI percentiles were 43.92 ± 27.84 in the thin/normal weight group and 41.36 ± 25.76 in the overweight/obese group at baseline with an increase for both groups during the two-year and six-year follow-up periods (Table 2). Increasing trends in the weekly duration of watching TV, playing computer/games and time spent at sports clubs were also observed after 2 and 6 years. In the subsample with accelerometer

Table 1 Demographic characteristics of the study population, stratified by weight status

	Main sample [a]		Subsample [b]	
	Thin/normal weight (*N* = 1627)	Overweight/obese (*N* = 381)	Thin/normal weight (*N* = 833)	Overweight/obese (*N* = 204)
Age (Mean, SD) [c]	6.02 (1.81)	6.64 (1.66)	6.35 (1.73)	6.83 (1.63)
Sex (N, %) [c]				
Boys	911 (83.8)	176 (16.2)	435 (83.2)	88 (16.8)
Girls	716 (77.7)	205 (22.3)	398 (77.4)	116 (22.6)
Family socio-economic status (N, %) [c]				
Low	142 (68.3)	66 (31.7)	69 (70.4)	29 (29.6)
Medium	893 (79.0)	238 (21.0)	500 (80.0)	125 (20.0)
High	592 (88.5)	77 (11.5)	264 (84.1)	50 (15.9)
Pubertal status (N, %) [d]				
Pre or early pubertal	343 (78.3)	95 (21.7)	174 (78.7)	47 (21.3)
Pubertal	180 (64.8)	98 (35.4)	128 (72.3)	49 (27.7)
Country (N, %) [c]				
Belgium	278 (93.0)	21 (7.0)	68 (93.1)	5 (6.9)
Estonia	274 (84.3)	51 (15.7)	183 (89.3)	22 (10.7)
Germany	472 (85.2)	82 (14.8)	310 (85.4)	53 (14.6)
Hungary	112 (88.9)	14 (11.1)	45 (83.3)	9 (16.7)
Italy	259 (60.7)	168 (39.3)	85 (52.5)	77 (47.5)
Spain	116 (81.1)	27 (18.9)	124 (78.5)	34 (21.5)
Sweden	116 (86.6)	18 (13.4)	18 (81.8)	4 (18.2)

[a]Main sample included participants with full information of self-reported physical activity and sedentary behaviour as well as co-variables. [b]Subsample included the participants with full information of accelerometer data as well as co-variables. [c]Data from baseline survey; [d]Data from six-year follow-up survey

Table 2 Baseline and changes of measurements over two-year and six-year follow-up

	Baseline		Two-year changes		Six-year changes	
	Thin/normal weight	Overweight/obese	Thin/normal weight	Overweight/obese	Thin/normal weight	Overweight/obese
	(N = 1627 [a]/833 [b])	(N = 381 [a]/204 [b])	(*N* = 1273 [a]/633 [b])	(*N* = 380 [a]/206 [b])	(*N* = 523 [a]/302 [b])	(*N* = 193 [a]/96 [b])
Anthropometric measures (Mean, SD) [c]						
Percentiles of bone stiffness index	43.92 (27.84)	41.36 (25.76)	3.86 (30.17)	4.60 (25.55)	8.08 (34.59) *	18.88 (33.46) *
Height z-score	0.37 (1.00) *	0.87 (1.02) *	0.04 (0.40)	0.06 (0.39)	0.20 (0.60) *	0.05 (0.68) *
Weight z-score	0.07 (0.87) *	2.00 (0.78) *	0.03 (0.38) *	0.19 (0.52) *	0.21 (0.66) *	0.43 (0.87) *
Reported healthy behaviour						
Watching TV/video/DVD (hours/week, Mean, SD) [c]	8.67 (5.00) *	9.74 (5.32) *	0.62 (4.57)	0.71 (5.13)	2.05 (6.41)	3.09 (7.57)
Playing computer/games (hours/week, Mean, SD) [c]	2.34 (3.39)	2.69 (3.58)	1.38 (3.58) *	2.17 (4.66) *	2.84 (5.83)	3.63 (7.16)
Sports clubs (hours/week, Mean, SD) [c]	1.13 (1.60)	1.32 (1.63)	0.72 (1.77)	0.56 (1.89)	1.41 (2.56)	0.90 (2.67)
Weight bearing sports (N, %) [d]						
Moderate or high mechanical loads	752 (79.5)	194 (20.5)	866 (76.5)	266 (23.5)	396 (73.5)	143 (26.5)
No or low mechanical loads	875 (82.4)	187 (17.6)	407 (78.1)	114 (21.9)	127 (71.8)	50 (28.2)
Accelerometer data (Mean, SD)						
Sedentary time (hours/day) [c]	4.46 (1.21) *	4.72 (1.26) *	0.70 (1.39)	0.61 (1.42)	2.31 (1.53)	2.41 (1.59)
Light physical activity (hours/day) [c]	6.37 (1.00)	6.39 (1.10)	−0.46 (1.14)	−0.57 (1.23)	−1.47 (1.28)	− 1.56 (1.29)
Moderate-to-vigorous physical activity (10 min/day) [c]	4.28 (2.21) *	3.57 (1.93) *	−0.23 (2.43)	− 0.45 (1.96)	0.10 (2.61)	− 0.50 (2.72)

[a] Sample size with full information of self-reported physical activity and sedentary behaviour as well as co-variables. [b] Sample size with full information of accelerometer data as well as co-variables. [c] Changes of values were the differences between follow-up and baseline measurements. [d] Changes of values were the percentages of reported moderate or high mechanical loads at baseline or follow-up, and no or low mechanical loads in both waves, respectively. * $p < 0.01$

data, there was a trend of increasing SB while LPA and MVPA slightly decreased over time.

Cross-sectional associations between SB, PA and SI percentiles

No statistically significant associations between self-reported and objectively measured SB, PA and SI were observed in the whole group at baseline. However, in thin/normal weight group, weekly duration of watching TV was inversely associated with SI percentiles (β = − 0.35, $p = 0.008$), while daily duration of objectively measured MVPA was positively associated with SI percentiles (β = 1.18, $p = 0.008$). Opposite but not statistically significant associations were observed for the overweight/obese group where SI percentiles were positively associated with the weekly duration of watching TV (β = 0.03, $p = 0.906$) while inversely associated with the daily duration of objectively measured MVPA (β = − 0.23, $p = 0.807$) (Table 3).

Longitudinal effects of SB and PA on changes in SI percentiles

In the whole group, change in time spent at sports clubs was positively associated with change in SI percentiles after 2 years (β = 1.28, $p = 0.001$); objectively measured MVPA at baseline was a strong predictor for change in SI percentiles (β = 2.77, $p < 0.001$). Similar effect sizes were observed after stratifying by weight status although the findings for overweight/obese group were not statistically significant. In contrast, weekly duration of watching TV at baseline (β = − 0.63, $p = 0.021$) and change after 2 years (β = − 0.63, $p = 0.022$) were inversely associated with change in SI percentiles only in overweight/obese group (Table 4).

Regarding the six-year follow-up, a statistically significant positive association between change in time spent at sports clubs and corresponding change in SI percentiles was observed only for the thin/normal weight group. As observed after 2 years, objectively measured MVPA at baseline also predicted change in SI percentiles after 6 years (β = 3.67, $p < 0.001$). In contrast to the slight effect of watching TV over the two-year period in overweight/obese group, we observed that six-year change in duration of playing computer/games was negatively associated with six-year change in SI percentiles (β = − 0.75, $p = 0.019$) (Table 5).

Table 3 Cross-sectional associations between sedentary behaviour, physical activity and bone stiffness index percentiles at baseline

	Whole group		Thin/normal weight group		Overweight/obese group	
	β (99%CI)	*p*-value	β (99%CI)	p-value	β (99%CI)	p-value
Main sample (self-reported data, N = 2008)						
Watching TV/video/DVD (hours/week)	− 0.23(− 0.53,0.06)	0.044	− 0.35(− 0.69,-0.01)	0.008	0.03(− 0.60,0.66)	0.906
Playing computer/games (hours/week)	− 0.004(− 0.49,0.48)	0.984	0.03(− 0.52,0.58)	0.883	0.03(− 0.96,1.01)	0.943
Sports clubs (hours/week)	1.00(− 0.40,2.39)	0.066	0.50(− 1.03,2.04)	0.398	2.68(− 0.58,5.95)	0.034
Weight bearing sports						
Moderate or high mechanical loads vs. No or low mechanical loads (reference)	− 1.80(−6.37,2.77)	0.310	− 0.13(−5.16,4.91)	0.948	−8.05(− 18.68,2.59)	0.051
Subsample (accelerometer data, N = 1037)						
Sedentary time (hours/day)	− 0.11(− 2.11,1.90)	0.891	1.01(− 1.29,3.31)	0.256	−3.85(−7.82,0.13)	0.013
Light physical activity (hours/day)	−0.94(− 3.06,1.19)	0.255	−1.46(− 3.90,0.99)	0.124	− 0.22(− 4.50,4.05)	0.893
Moderate-to-vigorous physical activity (10 min/day)	0.70(− 0.32,1.73)	0.077	1.18 (0.03,2.33)	0.008	− 0.23(− 2.62,2.17)	0.807

Adjusted for baseline age, sex, socio-economic status, daylight, height and weight z-score, country as a random effect

Effects of adherence to international PA and SB guidelines on SI percentiles

At baseline, 17.3% of the participants with accelerometer data adhered to the PA guideline of at least 1 h/d of objectively measured MVPA. When looking at the longitudinal data, only 6.3% adhered to the guideline at both baseline and two-year follow-up and 4.0% at both baseline and six-year follow-up. For participants who fulfilled the PA guideline at both time points, there was a higher increase of SI percentiles than for their counterparts with 10.39 units ($p = 0.002$) and 12.68 units ($p = 0.050$) over the two-year and six-year periods, respectively. Meanwhile, 88.8% of participants adhered to the screen time guideline of watching TV for no more than 14 h/w at baseline, 80.6% adhered to the guidelines at both baseline and two-year follow-up and 69.6% at both baseline and six-year follow-up. However, no associations were found between screen time guidelines and SI percentiles.

Discussion

Our results highlighted the importance of objectively measured MVPA at baseline for the development of a

Table 4 Longitudinal associations between sedentary behaviour, physical activity and bone stiffness index percentiles after 2 years

	Whole group		Thin/normal weight group		Overweight/obese group	
	β (99%CI)	*p*-value	β (99%CI)	p-value	β (99%CI)	p-value
Main sample (self-reported data,, N = 1653)						
Baseline watching TV/video/DVD (hours/week)	0.06(− 0.33,0.44)	0.712	0.28(− 0.18,0.74)	0.118	− 0.63(− 1.34,0.07)	0.021
Baseline playing computer/games (hours/week)	0.02(− 0.57,0.60)	0.943	− 0.12(− 0.81,0.57)	0.651	0.42(− 0.67,1.50)	0.318
Baseline sports clubs (hours/week)	1.07(− 0.23,2.36)	0.034	0.96(− 0.54,2.46)	0.098	1.03(− 1.54,3.60)	0.299
Change of watching TV/video/DVD (hours/week)	0.11(− 0.28,0.49)	0.468	0.36(−0.10,0.81)	0.042	−0.63(−1.35,0.08)	0.022
Change of playing computer/games (hours/week)	0.09(−0.35,0.54)	0.593	0.09(− 0.47,0.64)	0.688	0.32(− 0.41,1.04)	0.258
Change of sports clubs (hours/week)	1.28 (0.30,2.26)	0.001	1.29 (0.10,2.48)	0.005	1.04(−0.63,2.70)	0.110
Weight bearing sports						
Moderate or high mechanical loads vs. No or low mechanical loads (reference)	−0.69(−4.99,3.61)	0.678	0.32(− 4.76,5.41)	0.870	−3.13(−11.14,4.89)	0.313
Subsample (accelerometer data, N = 839)						
Baseline sedentary time (hours/day)	0.60(−1.77,2.96)	0.516	0.99(−1.86,3.85)	0.369	−0.39(−4.65,3.86)	0.811
Baseline light physical activity (hours/day)	−0.81(−3.50,1.89)	0.439	−1.60(−4.93,1.73)	0.214	0.16(−4.46,4.78)	0.927
Baseline moderate-to-vigorous physical activity (10 min/day)	2.77 (1.50,4.05)	< 0.001	2.97 (1.53,4.42)	< 0.001	2.85(−0.12,5.81)	0.013
Change of sedentary time (hours/day)	−0.85(−2.58,0.88)	0.205	−1.19(−3.25,0.87)	0.135	0.10(−3.09,3.29)	0.935
Change of light physical activity (hours/day)	−0.94(−3.03,1.16)	0.249	−1.45(−3.99,1.09)	0.140	0.42(−3.26,4.10)	0.768
Change of moderate-to-vigorous physical activity (10 min/day)	1.05(−0.09,2.18)	0.018	0.89(−0.39,2.16)	0.073	1.76(−0.88,4.40)	0.084

Adjusted for baseline age, sex, socio-economic status, daylight, bone stiffness index percentiles, height and weight z-scores, country as a random effect

Table 5 Longitudinal associations between sedentary behaviour, physical activity and bone stiffness index percentiles after 6 years

	Whole group		Thin/normal weight group		Overweight/obese group	
	β (99%CI)	p-value	β (99%CI)	p-value	β (99%CI)	p-value
Main sample (self-reported data, N = 716)						
Baseline watching TV/video/DVD (hours/week)	− 0.18(− 0.80,0.44)	0.444	−0.18(− 0.93,0.57)	0.531	−0.36(−1.50,0.77)	0.404
Baseline playing computer/games (hours/week)	0.24(−0.59,1.08)	0.448	0.33(−0.73,1.39)	0.420	−0.16(−1.51,1.20)	0.766
Baseline sports clubs (hours/week)	1.79(−0.16,3.74)	0.018	1.99(−0.29,4.27)	0.024	0.59(−3.20,4.38)	0.686
Change of watching TV/video/DVD (hours/week)	−0.16(− 0.61,0.29)	0.362	− 0.20(− 0.74,0.34)	0.335	−0.10(− 0.93,0.72)	0.744
Change of playing computer/games (hours/week)	−0.13(− 0.59,0.33)	0.472	0.11(− 0.45,0.67)	0.617	−0.75(−1.58,0.07)	0.019
Change of sports clubs (hours/week)	1.01(−0.10,2.12)	0.020	1.54 (0.23,2.85)	0.002	−0.46(−2.64,1.73)	0.588
Weight bearing sports						
Moderate or high mechanical loads vs. No or low mechanical loads (reference)	4.47(−2.29,11.22)	0.088	2.48(−5.39,10.36)	0.416	11.26(−1.69,24.22)	0.025
Subsample (accelerometer data, N = 398)						
Baseline sedentary time (hours/day)	−0.44(−4.00,3.13)	0.751	0.08(−4.19,4.35)	0.961	1.03(−5.82,7.88)	0.693
Baseline light physical activity (hours/day)	−2.70(−7.01,1.62)	0.106	−1.68(−6.76,3.41)	0.393	−5.13 (12.46,2.19)	0.068
Baseline moderate-to-vigorous physical activity (10 min/day)	3.67 (1.55,5.79)	< 0.001	3.49 (1.03,5.95)	< 0.001	4.94 (0.92,8.97)	0.002
Change of sedentary time (hours/day)	−0.18(−2.55,2.18)	0.840	0.16(−2.69,3.01)	0.884	−1.61(−5.51,2.28)	0.277
Change of light physical activity (hours/day)	−0.53(−3.78,2.71)	0.670	0.11(−3.70,3.92)	0.938	−2.18(−7.82,3.45)	0.309
Change of moderate-to-vigorous physical activity (10 min/day)	1.53(−0.04,3.10)	0.012	1.74(−0.10,3.59)	0.015	0.87(−2.09,3.83)	0.441

Adjusted for baseline age, sex, socio-economic status, daylight, bone stiffness index percentiles, height and weight z-scores and puberty at six-year follow-up, country as a random effect

healthy SI over two-year and six-year follow-up. These findings were robust and the effect sizes were consistent across weight statuses. We further demonstrated the benefit of adherence to established PA guidelines on long-term SI gain in children and adolescents, with those participating in objectively measured MVPA for at least 1 h per day having higher SI increases than their counterparts. These objectively measured results were supported by the comparable, albeit weak positive associations between self-reported time spent at sports clubs and changes in SI percentiles at two-year as well as six-year follow-ups. Regarding our assumption that being overweight/obese may be an important confounder, we observed controversial associations of screen-based SB with SI in the different weight strata. In general, the inversely cross-sectional associations between watching TV and SI were more pronounced in thin/normal weight children and adolescents than in overweight/obese ones. Nonetheless, in the longitudinal data, durations of specific screen-based SB were observed to be negatively associated with SI changes only in overweight/obese participants at the two-year and six-year follow-ups.

Even though the beneficial osteogenic effect of PA on bone mass accrual has already been well described in previous observational studies, most of the existing evidence so far mainly focused on BMC and/or BMD [51, 52]. Only a few cross-sectional studies have examined the associations between PA and QUS bone parameters. For example, Robinson et al. [53] demonstrated that time spent on moderate-to-high impact activities positively related to calcaneus SI in adolescent girls. Zulfarina et al. [54] reported that PA level, in terms of metabolic equivalent-minutes per week, was positively associated with three QUS parameters (i.e. BUA, SOS and SI) in adolescents. However, the PA levels in previous studies were mainly measured using different self-reported questionnaires, rendering it difficult to compare the results. Moreover, little is known about the optimal dose and intensity of PA and their sustainable effects on bone strength during growth. In our previous case-control study that was embedded in the IDEFICS study, we found that 30 min of objectively measured MVPA per day was not sufficient for an optimal SI [55]. A longitudinal study suggested that children in the upper quartile of objectively measured MVPA (approximated 1 h/d) had about 4 to 13% greater HR-pQCT-measured bone parameters at distal tibia compared to their peers in the lowest quartile (approximated 0.5 h/d) [56]. Our findings not only demonstrate that objectively measured MVPA rather than LPA using accelerometers is an important predictor of bone strength across weight strata, but also support the current opinion that adherence to the WHO recommendations for MVPA has a positive impact on bone strength. A recent systematic review

suggested that more than 80% of adolescents had insufficient physical activity globally illustrating the urgent need for further effective policies and intervention strategies in order to obtain optimal bone strength in children and adolescents [57].

Regarding self-reported PA at baseline, less than half of the parents reported that their children participated in a sports club. The average time spent at sports clubs was given as approximately 1 h per week. This indeed depicts reality, as children of that age do not commonly take part in sports club activities. Our finding of change in time spent at sports clubs rather than baseline time being more strongly related to change in SI was hence to be expected, as the participants were then 2 and 6 years older at the respective follow-ups. While no association between self-reported WBE and SI was observed in our study, the osteogenic effect of WBE has been reported in a review regarding school-based intervention programs, however, focusing mainly on jumping exercises [58]. Bone strength is thought to be less sensitive to light and moderate WBE among growing individuals [59]. As we did not have data describing the intensity of WBE in our study this may possibly explain why our results were non-significant. Further, as the self-reported sports club activities did not include WBE during leisure time, the effect of WBE on SI may have been underestimated.

Notably, the cross-sectional associations we observed did not match the longitudinal associations across weight strata, especially in self-reported screen-based SB. A possible explanation could be that the deleterious effects of watching TV on SI were covered by the stimulating effect of the mechanical loading exerted by weight status, which increased along with more screen time [60]. However, after relatively long-term exposure to screen-based SB, less SI gain still could occur in overweight/obese children. Potential direct and indirect mechanisms of weight status may influence the longitudinal relationship between screen-based SB and SI development. On the one hand, being sedentary may disrupt the bone formation-resorption balance due to lack of mechanical loading [61]. This detrimental effect may be stronger in overweight/obese individuals since they lose more mechanical loading exerted by body weight. On the other hand, overweight/obese children are also likely to spend more time watching TV or playing computer/games [62], eventually resulting in reduced SI. A similar observation was made in our data. Moreover, detrimental effects of screen-based SB appeared to be pattern-specific over time, with the durations of watching TV and playing computer/games observed to be inversely associated with SI gain at two-year and six-year follow-up, respectively. Although watching TV, the predominantly measured screen-based SB, represents the largest amount of screen-based SB for most children, recent studies suggest that computer use has increased dramatically over the years and has even replaced time spent watching TV, especially in adolescents [63, 64]. Marco et al. [17] also reported that non-study internet use rather than watching TV was negatively associated with whole body BMC in male adolescents. Our results are in line with this behavioural transition from childhood to adolescence and demonstrate that playing computer/games might present a higher risk factor for bone strength than watching TV as children get older.

The relationship between objectively measured SB and bone health during growth is still inconclusive. Results of a recent systematic review indicated the presence of a minor association between total SB and bone outcomes of the lower extremities in youth [65]. In the British Columbia Healthy Bones Study III cohort (HBSII), no associations were found between screen-based SB, total SB and bone architecture and strength in 9- to 20-year-old subjects at baseline [16], but total duration of objectively measured SB was found to be a negatively independent predictor in longitudinal analyses based on four annual follow-ups [56]. In contrast, we did not observe any longitudinal relationships between total duration of objectively measured SB and SI, except for a small cross-sectional inverse association in overweight/obese children. In our previous IDEFICS study with a larger cross-sectional sample, we found that total duration of objectively measured SB was negatively associated with SI in preschool and school children [26]. As only a fraction of the subgroup of participants in the present study who had accelerometer data could be linked in longitudinal data, we believe that the associations we detected did not reach the significance threshold due to the small sample size. Nevertheless, from our investigation, it still can be concluded that SB operationalised as screen time might be a valuable predictor of bone strength. However, the optimal dose of SB as well as of specific screen-based SB on bone strength needs to be further investigated in longitudinal studies and interventions.

Our study has several strengths and limitations. To our best knowledge, this is the first longitudinal study to present the associations between SB, PA and QUS parameters using repeatedly measured data among European children and adolescents. Moreover, in addition to self-reported questionnaires, we investigated SB and PA with objective measurements in a relatively large subsample and thereby acquired more precise information regarding the intensity and quantification of activity levels. Additionally, we were able to identify differential associations of SI across weight strata, which helped provide better insight into the role of weight status in these associations. While we were able to collect some

objective data, the fact that the data was only available for a subsample whose size diminished considerably at each follow-up is a limitation that most likely led to the lack of statistical power, and our results may not be generalised for the whole population. However, as the subsample's baseline mean of SI percentiles (41.19 ± 26.58) and rate of overweight/obesity (19.7%) were comparable to that of the main sample (43.44 ± 27.47 and 19.0%, respectively), we believe that this reduces the potential for bias. Second, the imprecision of the self-reported WBE (only based on sports club activities) may have led to underestimations regarding cross-sectional and longitudinal effects of WBE on SI. Third, the comparability of ActiTrainer with other Actigraph accelerometers in previous validation studies is still inconclusive. However, we additionally included a confounder to account for different measures induced by the use of GT1M and ActiTrainer, which did not change our final results. Therefore, we are convinced that our data collection by different types of accelerometers provides comparable PA values. Finally, we did not include nutritional variables such as calcium intake. Instead, we considered weekly frequency of milk and dairy products consumption from a food frequency questionnaire as a proxy. Since we did not observe an influential effect on our final results, we did not consider this variable further to avoid having to exclude more participants, which would have reduced the sample size considerably.

Conclusions

In summary, our results demonstrated that objectively measured MVPA is an important predictor of bone strength across weight strata. Meeting the MVPA recommendation of 1 h per day maintained the beneficial effect on bone strength during the six-year observational period. On the other hand, the increasing durations of screen-based SB might be risk factors for SI development, especially in overweight/obese participants. Finally, bone health improving interventions should promote high intensive exercises and also focus on the reduction of screen-based SB, particularly when targeting overweight/obese individuals.

Abbreviations

PBM: Peak bone mass; PA: Physical activity; SB: Sedentary behaviour; WBE: Weight-bearing exercises; WHO: World Health Organization; LPA: Light physical activity; MVPA: Moderate-to-vigorous physical activity; QUS: Quantitative ultrasound; SI: Stiffness index; BUA: Broadband ultrasound attenuation; SOS: Speed of sound; BMC: Bone mineral content; BMD: Bone mineral density; DXA: Dual energy X-ray absorptiometry; BMI: Body mass index; IOTF: International Obesity Task Force; CPM: Counts per minute; SES: Socio-economic status; ISCED: International Standard Classification of Education; SD: Standard deviation; CI: Confidence interval

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Authors' contributions

L.C., W.A. and A.H. conceptualised and designed the study. L.C., H.P. and A.H. analysed and interpreted the data. W.A., F.L., T.V., C.C., D.M., G.E., N.M., L.M., A.P. and Y.P. contributed to coordination and data collection. L.C. was a major contributor in writing the manuscript. All authors read and improved the manuscript and approved the final manuscript.

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Availability of data and materials

The datasets generated and analysed during the current study are not publicly available because this study is based on highly sensitive data collected in young children. But interested researchers can contact the IDEFICS and I. Family consortia (http://www.ideficsstudy.eu/Idefics/ and http://www.ifamilystudy.eu/) to discuss possibilities for data access.

Ethics approval and consent to participate

All parents and their children above 12 years old signed informed consent, while younger children gave oral consent prior to the examinations in addition to the signed parental consent. Ethical approval was obtained from the ethics committees for all participating centres in each country: Ethics Committee, University Hospital, Gent, Belgium; Cyprus National Bioethics Committee, Nicosia, Cyprus; Tallinn Medical Research Ethics Committee, Tallinn, Estonia; Ethics Committee of the University of Bremen, Bremen, Germany; Egészségügyi Tudományos Tanács, Pécs, Hungary; Azienda Sanitaria Locale Avellino Comitato Etico, Avellino, Italy; Regionala Etikprövningsnämnden i Göteborg, Gothenburg, Sweden; Comité Ético de Investigación Clínica de Aragón, Zaragoza, Spain.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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References

1. Bachrach LK. Acquisition of optimal bone mass in childhood and adolescence. Trends Endocrinol Metab. 2001;12(1):22–8.

2. Nguyen TV, Maynard LM, Towne B, Roche AF, Wisemandle W, Li J, et al. Sex differences in bone mass acquisition during growth: the Fels longitudinal study. J Clin Densitom. 2001;4(2):147–57.
3. Mitchell JA, Cousminer DL, Zemel BS, Grant SF, Chesi A. Genetics of pediatric bone strength. Bonekey Rep. 2016;5:823.
4. Gordon CM, Zemel BS, Wren TA, Leonard MB, Bachrach LK, Rauch F, et al. The determinants of peak bone mass. J Pediatr. 2017;180:261–9.
5. Julian-Almarcegui C, Gomez-Cabello A, Huybrechts I, Gonzalez-Aguero A, Kaufman JM, Casajus JA, et al. Combined effects of interaction between physical activity and nutrition on bone health in children and adolescents: a systematic review. Nutr Rev. 2015;73(3):127–39.
6. Seeman E. Reduced bone formation and increased bone resorption: rational targets for the treatment of osteoporosis. Osteoporos Int. 2003;14(Suppl 3): S2–8.
7. Behringer M, Gruetzner S, McCourt M, Mester J. Effects of weight-bearing activities on bone mineral content and density in children and adolescents: a meta-analysis. J Bone Miner Res. 2014;29(2):467–78.
8. Tan VP, Macdonald HM, Kim S, Nettlefold L, Gabel L, Ashe MC, et al. Influence of physical activity on bone strength in children and adolescents: a systematic review and narrative synthesis. J Bone Miner Res. 2014;29(10): 2161–81.
9. Konstabel K, Veidebaum T, Verbestel V, Moreno LA, Bammann K, Tornaritis M, et al. Objectively measured physical activity in European children: the IDEFICS study. Int J Obes. 2014;38(Suppl 2):S135–43.
10. Marques A, Gaspar de Matos M. Adolescents' physical activity trends over the years: a three-cohort study based on the health behaviour in school-aged children (HBSC) Portuguese survey. BMJ Open. 2014;4(9):e006012.
11. Sigmund E, Sigmundova D, Badura P, Kalman M, Hamrik Z, Pavelka J. Temporal trends in overweight and obesity, physical activity and screen time among Czech adolescents from 2002 to 2014: a National Health Behaviour in school-aged children study. Int J Environ Res Public Health. 2015;12(9):11848–68.
12. Carson V, Hunter S, Kuzik N, Gray CE, Poitras VJ, Chaput JP, et al. Systematic review of sedentary behaviour and health indicators in school-aged children and youth: an update. Appl Physiol Nutr Metab. 2016;41(6 Suppl 3): S240–65.
13. Verloigne M, Van Lippevelde W, Maes L, Yildirim M, Chinapaw M, Manios Y, et al. Self-reported TV and computer time do not represent accelerometer-derived total sedentary time in 10 to 12-year-olds. Eur J Pub Health. 2013; 23(1):30–2.
14. Brug J, van Stralen MM, Te Velde SJ, Chinapaw MJ, De Bourdeaudhuij I, Lien N, et al. Differences in weight status and energy-balance related behaviors among schoolchildren across Europe: the ENERGY-project. PLoS One. 2012;7(4):e34742.
15. Sioen I, Michels N, Polfliet C, De Smet S, D'Haese S, Roggen I, et al. The influence of dairy consumption, sedentary behaviour and physical activity on bone mass in Flemish children: a cross-sectional study. BMC Public Health. 2015;15:717.
16. Gabel L, McKay HA, Nettlefold L, Race D, Macdonald HM. Bone architecture and strength in the growing skeleton: the role of sedentary time. Med Sci Sports Exerc. 2015;47(2):363–72.
17. Gracia-Marco L, Rey-Lopez JP, Santaliestra-Pasias AM, Jimenez-Pavon D, Diaz LE, Moreno LA, et al. Sedentary behaviours and its association with bone mass in adolescents: the HELENA cross-sectional study. BMC Public Health. 2012;12:971.
18. McVeigh JA, Zhu K, Mountain J, Pennell CE, Lye SJ, Walsh JP, et al. Longitudinal trajectories of television watching across childhood and adolescence predict bone mass at age 20 years in the Raine study. J Bone Miner Res. 2016;31(11):2032–40.
19. Winther A, Ahmed LA, Furberg AS, Grimnes G, Jorde R, Nilsen OA, et al. Leisure time computer use and adolescent bone health--findings from the Tromso study, fit futures: a cross-sectional study. BMJ Open. 2015;5(6): e006665.
20. NCD Risk Factor Collaboration. Worldwide trends in body-mass index, underweight, overweight, and obesity from 1975 to 2016: a pooled analysis of 2416 population-based measurement studies in 128.9 million children, adolescents, and adults. Lancet. 2017;390(10113):2627–42.
21. van Leeuwen J, Koes BW, Paulis WD, van Middelkoop M. Differences in bone mineral density between normal-weight children and children with overweight and obesity: a systematic review and meta-analysis. Obes Rev. 2017;18(5):526–46.
22. Wetzsteon RJ, Petit MA, Macdonald HM, Hughes JM, Beck TJ, McKay HA. Bone structure and volumetric BMD in overweight children: a longitudinal study. J Bone Miner Res. 2008;23(12):1946–53.
23. Tanaka C, Janssen X, Pearce M, Parkinson K, Basterfield L, Adamson A, et al. Bidirectional associations between adiposity, sedentary behavior, and physical activity: a longitudinal study in children. J Phys Act Health. 2018;7:1–9.
24. Ahrens W, Bammann K, Siani A, Buchecker K, De Henauw S, Iacoviello L, et al. The IDEFICS cohort: design, characteristics and participation in the baseline survey. Int J Obes. 2011;35(Suppl 1):S3–15.
25. Ahrens W, Siani A, Adan R, De Henauw S, Eiben G, Gwozdz W, et al. Cohort Profile: The transition from childhood to adolescence in European children-how I. Family extends the IDEFICS cohort. Int J Epidemiol. 2017;46(5):1394–5j.
26. Herrmann D, Buck C, Sioen I, Kouride Y, Marild S, Molnar D, et al. Impact of physical activity, sedentary behaviour and muscle strength on bone stiffness in 2-10-year-old children-cross-sectional results from the IDEFICS study. Int J Behav Nutr Phys Act. 2015;12:112.
27. Baroncelli GI. Quantitative ultrasound methods to assess bone mineral status in children: technical characteristics, performance, and clinical application. Pediatr Res. 2008;63(3):220–8.
28. Alwis G, Rosengren B, Nilsson JA, Stenevi-Lundgren S, Sundberg M, Sernbo I, et al. Normative calcaneal quantitative ultrasound data as an estimation of skeletal development in Swedish children and adolescents. Calcif Tissue Int. 2010;87(6):493–506.
29. Torres-Costoso A, Vlachopoulos D, Ubago-Guisado E, Ferri-Morales A, Cavero-Redondo I, Martinez-Vizcaino V, et al. Agreement between dual-energy X-ray absorptiometry and quantitative ultrasound to evaluate bone health in adolescents: the PRO-BONE study. Pediatr Exerc Sci. 2018;30(4): 466–73.
30. Trimpou P, Bosaeus I, Bengtsson BA, Landin-Wilhelmsen K. High correlation between quantitative ultrasound and DXA during 7 years of follow-up. Eur J Radiol. 2010;73(2):360–4.
31. Sioen I, Mouratidou T, Herrmann D, De Henauw S, Kaufman JM, Molnar D, et al. Relationship between markers of body fat and calcaneal bone stiffness differs between preschool and primary school children: results from the IDEFICS baseline survey. Calcif Tissue Int. 2012;91(4):276–85.
32. Herrmann D, Intemann T, Lauria F, Marild S, Molnar D, Moreno LA, et al. Reference values of bone stiffness index and C-terminal telopeptide in healthy European children. Int J Obes. 2014;38(Suppl 2):S76–85.
33. Stomfai S, Ahrens W, Bammann K, Kovacs E, Marild S, Michels N, et al. Intra- and inter-observer reliability in anthropometric measurements in children. Int J Obes. 2011;35(Suppl 1):S45–51.
34. Cole TJ, Freeman JV, Preece MA. British 1990 growth reference centiles for weight, height, body mass index and head circumference fitted by maximum penalized likelihood. Stat Med. 1998;17(4):407–29.
35. Cole TJ, Lobstein T. Extended international (IOTF) body mass index cut-offs for thinness, overweight and obesity. Pediatr Obes. 2012;7(4):284–94.
36. Tremblay MS, Carson V, Chaput JP, Connor Gorber S, Dinh T, Duggan M, et al. Canadian 24-hour movement guidelines for children and youth: an integration of physical activity, sedentary behaviour, and sleep. Appl Physiol Nutr Metab. 2016;41(6 Suppl 3):S311–27.
37. American Academy of Pediatrics. Committee on Public E. American Academy of Pediatrics: children, adolescents, and television. Pediatrics. 2001; 107(2):423–6.
38. Lee KY, Macfarlane DJ, Cerin E. Comparison of three models of actigraph accelerometers during free living and controlled laboratory conditions. Eur J Sport Sci. 2013;13(3):332–9.
39. Santos-Lozano A, Santin-Medeiros F, Cristi-Montero C, Jaen-Jimenez R, Casajus JA, Garatachea N. GT1M, GT3X and ActiTrainer counts comparison during standardized activities in young, adults and older adults. Nutr Hosp. 2016;33(3):280.
40. Sasaki JE, John D, Freedson PS. Validation and comparison of ActiGraph activity monitors. J Sci Med Sport. 2011;14(5):411–6.
41. Robusto KM, Trost SG. Comparison of three generations of ActiGraph activity monitors in children and adolescents. J Sports Sci. 2012;30(13):1429–35.
42. Trost SG, Loprinzi PD, Moore R, Pfeiffer KA. Comparison of accelerometer cut points for predicting activity intensity in youth. Med Sci Sports Exerc. 2011;43(7):1360–8.
43. Buck C, Eiben G, Lauria F, Konstabel K, Page A, Ahrens W, et al. Urban Moveability and physical activity in children: longitudinal results from the IDEFICS and I. Family cohort. Int J Behav Nutr Phys Act. 2019; 16(1):128.

44. Harvey NC, Cole ZA, Crozier SR, Kim M, Ntani G, Goodfellow L, et al. Physical activity, calcium intake and childhood bone mineral: a population-based cross-sectional study. Osteoporos Int. 2012;23(1):121–30.
45. WHO. Global recommendations on physical activity for health. 2010.
46. UNESCO. International Standard Classification of Education 2010. http://www.uis.unesco.org/Education/Pages/international-standardclassification-of-education.aspx.
47. Harries M, Hawkins S, Hacking J, Hughes I. Changes in the male voice at puberty: vocal fold length and its relationship to the fundamental frequency of the voice. J Laryngol Otol. 1998;112(5):451–4.
48. Harries ML, Walker JM, Williams DM, Hawkins S, Hughes IA. Changes in the male voice at puberty. Arch Dis Child. 1997;77(5):445–7.
49. Lawn RB, Lawlor DA, Fraser A. Associations between maternal Prepregnancy body mass index and gestational weight gain and Daughter's age at menarche: the Avon longitudinal study of parents and children. Am J Epidemiol. 2018;187(4):677–86.
50. Moon RJ, Davies JH, Cooper C, Harvey NC. Vitamin D, and maternal and child health. Calcif Tissue Int. 2020;106(1):30–46.
51. Heidemann M, Molgaard C, Husby S, Schou AJ, Klakk H, Moller NC, et al. The intensity of physical activity influences bone mineral accrual in childhood: the childhood health, activity and motor performance school (the CHAMPS) study, Denmark. BMC Pediatr. 2013;13:32.
52. Janz KF, Gilmore JM, Levy SM, Letuchy EM, Burns TL, Beck TJ. Physical activity and femoral neck bone strength during childhood: the Iowa bone development study. Bone. 2007;41(2):216–22.
53. Robinson ML, Winters-Stone K, Gabel K, Dolny D. Modifiable lifestyle factors affecting bone health using calcaneus quantitative ultrasound in adolescent girls. Osteoporos Int. 2007;18(8):1101–7.
54. Zulfarina MS, Sharif R, Syarifah-Noratiqah SB, Sharkawi AM, Aqilah-Sm ZS, Mokhtar SA, et al. Modifiable factors associated with bone health in Malaysian adolescents utilising calcaneus quantitative ultrasound. PLoS One. 2018;13(8):e0202321.
55. Herrmann D, Pohlabeln H, Gianfagna F, Konstabel K, Lissner L, Marild S, et al. Association between bone stiffness and nutritional biomarkers combined with weight-bearing exercise, physical activity, and sedentary time in preadolescent children. A case-control study. Bone. 2015;78:142–9.
56. Gabel L, Macdonald HM, Nettlefold L, McKay HA. Physical activity, sedentary time, and bone strength from childhood to early adulthood: a mixed longitudinal HR-pQCT study. J Bone Miner Res. 2017;32(7):1525–36.
57. Guthold R, Stevens GA, Riley LM, Bull FC. Global trends in insufficient physical activity among adolescents: a pooled analysis of 298 population-based surveys with 1.6 million participants. Lancet Child Adolesc Health. 2020;4(1):23–35.
58. Nguyen VH. School-based exercise interventions effectively increase bone mineralization in children and adolescents. Osteoporos Sarcopenia. 2018;4(2):39–46.
59. Sayers A, Mattocks C, Deere K, Ness A, Riddoch C, Tobias JH. Habitual levels of vigorous, but not moderate or light, physical activity is positively related to cortical bone mass in adolescents. J Clin Endocrinol Metab. 2011;96(5):E793–802.
60. Keane E, Li X, Harrington JM, Fitzgerald AP, Perry IJ, Kearney PM. Physical activity, sedentary behavior and the risk of overweight and obesity in school-aged children. Pediatr Exerc Sci. 2017;29(3):408–18.
61. Tremblay MS, Colley RC, Saunders TJ, Healy GN, Owen N. Physiological and health implications of a sedentary lifestyle. Appl Physiol Nutr Metab. 2010;35(6):725–40.
62. Stierlin AS, De Lepeleere S, Cardon G, Dargent-Molina P, Hoffmann B, Murphy MH, et al. A systematic review of determinants of sedentary behaviour in youth: a DEDIPAC-study. Int J Behav Nutr Phys Act. 2015;12:133.
63. Huhman M, Lowry R, Lee SM, Fulton JE, Carlson SA, Patnode CD. Physical activity and screen time: trends in U.S. children aged 9-13 years, 2002-2006. J Phys Act Health. 2012;9(4):508–15.
64. Sigmundova D, Sigmund E, Bucksch J, Badura P, Kalman M, Hamrik Z. Trends in screen time Behaviours in Czech schoolchildren between 2002 and 2014: HBSC study. Cent Eur J Public Health. 2017;25(Suppl 1):S15–20.
65. Koedijk JB, van Rijswijk J, Oranje WA, van den Bergh JP, Bours SP, Savelberg HH, et al. Sedentary behaviour and bone health in children, adolescents and young adults: a systematic review. Osteoporos Int. 2017;28(9):2507–19.

Publisher's Note

Paper 3

Cross-sectional and longitudinal associations between sleep duration, sleep quality and bone stiffness in European children and adolescents

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ORIGINAL ARTICLE

Cross-sectional and longitudinal associations between sleep duration, sleep quality, and bone stiffness in European children and adolescents

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Abstract

Summary In this large perspective cohort among European children and adolescents, we observed that daytime napping was positively associated with bone stiffness, while short or long sleep duration combined with poor sleep quality was associated with less bone stiffness. Our findings are important for obtaining optimal bone stiffness in childhood.

Introduction To examine the cross-sectional and longitudinal associations between sleep duration, sleep quality, and bone stiffness index (SI) in European children and adolescents.

Methods Four thousand eight hundred seventy-one children aged 2–11 years from the IDEFICS study and 861 children aged 6–15 years from the subsequent I.Family study were included. Sleep duration (i.e., nocturnal sleep and daytime napping) and sleep quality (i.e., irregularly bedtime routine, have difficulty falling asleep and trouble getting up in the morning) were reported by self-administrated questionnaires. Nocturnal sleep duration was converted into age-specific z-scores, and total sleep duration was classified into short, adequate, and long based on the National Sleep Recommendation. Calcaneal SI of both feet were measured using quantitative ultrasound. Linear mixed-effects models with country as a random effect were used, with adjustments for sex, age, pubertal status, family socioeconomic status, physical activity, screen time, body mass index, and daylight duration.

Results Nocturnal sleep duration z-scores were positively associated with SI percentiles among participants with adequate sleep duration at baseline. Moreover, the positive association between daytime napping and SI percentiles was more pronounced in participants with adequate sleep duration at baseline, while at 4-year follow-up was more pronounced in participants with short sleep duration. In addition, extreme sleep duration at baseline predicted lower SI percentiles after 4 years in participants with poor sleep quality.

Conclusion The positive associations between nocturnal sleep, daytime napping and SI depended on total sleep duration. Long-term detrimental effect of extreme sleep duration on SI only existed in individuals with poor sleep quality.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00198-020-05753-x.

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Keywords Bone stiffness index · Child health · Sleep duration · Sleep quality

Introduction

Sleep duration among European children and adolescents decreased over the last decades, as reported by a systematic review in 2012 [1]. Furthermore, the transition from childhood to adolescence is often accompanied by altered sleep habits, such as irregular bedtime routines and evening-type circadian preference [2]. Researchers have also raised concerns that difficulties in initiating and maintaining sleep are occurring in youth [3, 4], and poor quality of sleeping states (e.g., difficulties falling asleep) and waking states (e.g., trouble getting up in the morning) are equally relevant to health. Even though daytime napping could compensate in part for less nocturnal sleep duration and poor sleep quality, it may also lead to excessive sleep duration as well as less efficient sleep at night time [5]. To date, poor sleep, in terms of extreme duration and poor quality, has been linked to increased risks of poor mental health, obesity, and cardio-metabolic risk among children and adolescents [6].

Emerging evidence has also proposed hypotheses regarding the health effects of sleep on bone metabolism. One potential pathway is the modulation of biological factors related to circadian rhythms [7], sympathetic nervous system [8], and pro-inflammation [9]. Another potential explanation may be the behavioral changes caused by poor sleep such as unhealthy eating behaviors, less physical activity (PA), and more sedentary behaviors (SB) [10], which may further exert detrimental effects on bone accrual. In fact, a number of studies among adults have supported these hypotheses. A recent meta-analysis among middle-aged and elderly adults suggested a U-shaped dose-response with the pooled odds ratios (ORs) of osteoporosis risk of 1.03 (95% CI 1.01–1.06) for each 1-h sleep reduction among individuals with shorter sleep duration (< 8 h/day), and 1.01 (95% CI 1.00–1.02) for each 1-h sleep increment among individuals with longer sleep duration (> 8 h/day) [11]. Two observational studies suggested that poor sleep quality was associated with adulthood osteoporosis measured using Dual-energy X-ray absorptiometry (DXA) [12] and quantitative ultrasound (QUS) [13]. Considering that a large proportion of bone accrual increases during childhood until peak bone mass is achieved during young adulthood [14], the role of sleep on bone health is probably more pronounced in early life. However, few studies have been conducted in children and adolescents, and reported inconsistent results with positive [15, 16] or no associations [17] of sleep duration with bone mineral content (BMC) and bone mineral density (BMD). Besides, little attention has been paid to sleep quality as well as its interrelated effects with nocturnal sleep and daytime napping on bone health.

A better understanding of the relationship between sleep and bone accrual could be helpful to obtain optimal bone strength, which is also considered to be an important prevention strategy for osteoporosis fracture in later life. In order to fill the gaps in previous studies, we aimed to investigate cross-sectional and longitudinal associations of nocturnal sleep duration, daytime napping, and sleep quality with bone stiffness index (SI) measured using QUS from a large population-based cohort of children and adolescents, and to further explore the interactive effects between total sleep duration and sleep quality on SI.

Methods

Study design and population

The IDEFICS/I.Family cohort is a multicentre population-based study which was conducted in eight European countries: Belgium, Cyprus, Estonia, Germany, Hungary, Italy, Spain, and Sweden. The main aim of this cohort was to investigate and prevent diet- and lifestyle-related diseases and disorders among European children and adolescents. The baseline survey (IDEFICS study) was conducted between September 2007 and May 2008, two or more communities in each country whose socio-demographic profile and infrastructure were similar and typical for their region were selected. In total, 16,229 children aged 2 to 9 years participated and fulfilled the inclusion criteria. Two follow-up examinations were further conducted with similar examination modules after 2 years (IDEFICS study) and 6 years (I.Family study) at 2009/2010 and 2013/2014, respectively. The study was conducted according to the standards of the Declaration of Helsinki. Ethical approval was obtained from the ethics committees by all eight study centers. Before children entered the study, parents provided written informed consent. Additionally, children aged 12 years and older gave simplified written consent and children younger than 12 years gave oral consent before each examination. More details on the study design of the IDEFICS/I.Family cohort were published previously [18].

We included data from who participated in the 2009/2010 (in the following referred to as baseline) and 2013/2014 surveys (in the following referred to as follow-up) of the IDEFICS/I.Family cohort, since the sleep variables of interest were only assessed at these two time points. QUS

measurements were conducted as an optional module in each survey, with 6886 participants at baseline (50.6%) and 2892 participants at follow-up (30.3%), respectively. There were no significant differences between participants and non-participants for main demographic characteristics (i.e., sex, age, and family socioeconomic status (SES)). We further excluded children and adolescents who (1) had implausible QUS measurements, i.e., absolute difference of SI between the right and left foot exceeded the 97th percentile (39 units) based on total 8280 measurements; (2) had an indication of impaired bone health at baseline, i.e., with disease or receiving medical treatments affecting the bone; (3) had implausible nocturnal sleep duration, i.e., more than 16 h per night or less than 5 h per night; and (4) had incomplete data of covariates, i.e., PA, SB, and pubertal status. Finally, a total of 4871 children at baseline were eligible for the cross-sectional analysis. Of these, 861 participants with complete information at both baseline and follow-up were included in the longitudinal analysis.

Questionnaires

General questionnaires related to lifestyle behaviors were collected in the IDEFICS/I.Family cohort. Parents were asked to complete the questionnaires on behalf of their children younger than 12 years old, while for older children and adolescents, the questionnaire was self-reported.

Sleep characteristics

The duration of nocturnal sleep and daytime napping (hours and min) at weekdays/school days and weekend days/vacations was separately recorded. The average of daily nocturnal sleep duration was calculated for each child as follows: (nocturnal sleep duration on weekdays/school days × 5 + nocturnal sleep duration on weekend days/vacations × 2) / 7. The value of nocturnal sleep duration for each child was further transformed to an age-specific z-score based on the reference population from I.Family study, in order to consider both children and adolescents. The maximum number of observations with plausible information on age and nocturnal sleep duration was included to obtain the best possible estimates; the years of age were used as categories to calculate age-specific means and standard deviations. Analogously, the average of daily napping time was calculated and expressed as 10 min/day in order to better interpret the regression coefficients.

We further calculated the total sleep duration by adding up the nocturnal sleep and daytime napping. According to the sleep recommendation from the National Sleep Foundation (NSF) [19], we used the definition of short and long sleep duration as < 10 h/day and ≥ 13 h/day for pre-school children aged 2 to < 6 years, < 9 h/day and ≥ 11 h/day for primary school children aged 6 to < 12 years, < 8 h/day and ≥ 10 h/day for adolescents aged 12 to 15 years, respectively.

Additionally, participants were asked to report their typical sleeping habits and daytime condition as follows: (1) do you have a regular bedtime routine (yes = 1 and no = 0); (2) do you have trouble getting up in the morning (yes = 0 and no = 1); and (3) do you have difficulty falling asleep (yes = 0 and no = 1). Similar items were used previously in other large population-based study [20]. We calculated a cumulative score as the sum of these three items (range from 0 to 3); individuals scoring 3 were considered as having good sleep quality.

Physical activity and sedentary behavior

PA level of the participants was recorded as weekly duration of participation in sports clubs. They were asked to report whether the child was a member of a sports club (yes or no); if the answer was yes, then they had to report how many hours and minutes per week. The weekly time spent at sports clubs was calculated by adding hours and minutes and expressed as hours per week (h/w). SB level of the participants was recorded as daily duration of total screen time. Screen time was calculated from reported usual duration of the child watching TV/videos/DVDs and playing computer/game console on a normal weekday and weekend day. For both questions, six response categories were offered and converted into the following scoring system: not at all = 0, < 30 min = 1, < 1 h = 2, 1– < 2 h = 3, 2–3 h = 4, and > 3 h = 5. Each screen-based SB was calculated separately for weekdays and weekend days by adding up the converted responses of questions as follows: screen-based SB on weekdays/school days × 5 + screen-based SB on weekend days/vacations × 2. The weekly duration of screen time was the total duration of these two screen-based SB and expressed as hours per week (h/w).

Other covariates

The age, sex, and SES of participants were obtained from parental questionnaire. The highest education of parents was obtained as a proxy indicator for SES according to the International Standard Classification of Education (ISCED) (low: ISCED levels 0–2; medium: ISCED levels 3–4; high: ISCED levels 5 and higher) [21]. The pubertal status was self-reported by children aged 8 years and older, and was defined as pre-pubertal or pubertal based on voice change in boys and first menstrual period in girls [16]. Sunlight exposure accounts for the main source of vitamin D synthesis [22]; therefore, mean daylight duration (± 0.1 h) for each examination month in each location was calculated using astronomical tables as a proxy for vitamin D level.

Bone stiffness index

SI was measured on the left and right calcaneus using QUS (Achilles Lunar Insight TM GE Healthcare, Milwaukee, WI, USA); the reliability study and the methodology of QUS device have previously been described in detail [23]. The measurements and quality control were performed by trained nurses based on the standard operating procedures provided by the manufacturer. The foot was positioned using two different sizes of adapters for participants to keep their calcaneus properly. Before recording each measurement, the preview image of the calcaneus and the region of interest were required to display, in order to avoid measurement error caused by incorrect locations, e.g., the growth plate. The SI was calculated automatically as a percentage (units) based on normalized and scaled values of broadband ultrasound attenuation (BUA, dB/MHz) and speed of sound (SOS, m/s) according to: BSI = (0.67*BUA) + (0.28*SOS) − 420. The value of BUA represents the spatial orientation of the bone trabeculae and increases with greater trabecular complexity. The value of SOS represents the velocity of sound traveling through the bone and increases with greater structures density, and the combination of SOS and BUA was slightly better at predicting bone strength than either parameter alone [24]. The mean SI of the left and right calcaneus measurements was calculated and used in the statistical analysis. For each individual, the SI percentile was calculated additionally as outcome according to age, sex, and height based on the IDEFICS/I.Family reference population. The processing method and the first descriptive results of SI percentile values in the IDEFICS study can be found elsewhere [23], and we additionally provided the sex-specific reference curves for SI percentiles by age for average children based on the 50th height percentile in the IDEFICS/I.Family study in Online Resource (Figure S1).

Anthropometrics measurements

Physical examinations including body weight and height were measured by trained nurses based on the standard operating procedures. Weight (kg) was measured to the nearest 0.1 kg using the Tanita scale (BC420 MA for children and BC418 MA for adolescents, Tanita Europe GmbH, Sindelfingen, Germany). Height (cm) was measured to the nearest 0.1 cm using the calibrated stadiometer (Seca 225/213 stadiometer, Birmingham, UK). All examinations were in light clothing without shoes. Body mass index (BMI) was calculated as weight (kg) divided by height (m) squared; the values were transformed to age- and sex-specific z-scores based on Cole et al. [25]

Statistical methods

Associations between sleep duration, sleep quality, and SI were investigated using linear mixed-effects models, and a random effect for country was added to account for cluster effects. All models were adjusted for age, sex, family SES, daylight duration, and BMI z-scores in each survey. The exposures of sleep duration at baseline and/or follow-up were included as continuous variables (i.e., nocturnal sleep duration z-scores and daytime napping duration) and a dichotomous variable (i.e., fulfilling the sleep recommendation or not) in separate models, to additionally investigate the benefit of meeting sleep recommendation on SI percentiles. The exposure of sleep quality at baseline and/or follow-up was included as a dichotomous variable (i.e., good and poor). The outcome for cross-sectional analyses was baseline SI percentile, with additional adjustments for baseline duration of PA and SB. The outcome for longitudinal analyses was SI percentile at follow-up, with additional adjustments for SI percentile at baseline, pubertal status at follow-up, and average durations of PA and SB at both surveys. Since sleep duration and sleep quality may have interactive effects on SI, we further stratified the whole group by total sleep duration and quality, to investigate the different effects of interests across stratifications.

All the statistical analyses were carried out with the Statistical Analysis System (SAS) software package (Version 9.4; SAS Institute, Cary, NC). Regression coefficients (β), 95% confidence intervals (95%CIs), and p values were calculated. The significance level was set at $\alpha = 0.05$ (2-sided tests); multiple testing was further adjusted and cited in footnotes if p values exceed 0.05 according to Holm's sequential Bonferroni procedure [26].

Results

Descriptive analyses

At baseline, 4871 participants consist of 742 pre-school children and 4129 primary school children were included in cross-sectional analyses. Of these, 861 participants consist of 352 primary school children and 509 adolescents from 5 participating centers were further included in longitudinal analyses after a 4-year follow-up. The proportion of boys (47.0% vs. 50.5%, $p = 0.06$) and mean age (8.28 years vs. 8.20 years, p = 0.19) of longitudinal analytic sample were comparable to children who did not provide follow-up data, but less participants were classified as low (8.4% vs. 9.1%) and high SES (30.0% vs. 38.1%), $p < 0.001$. More details of demographic characteristics are shown in Table 1; additional results of Pearson's correlations and 95%CIs between co-variables, sleep exposures, and bone stiffness index can be found in Online Resource (Table S1).

Table 1 Descriptive characteristics of the study population

	Baseline			Follow-up		
	Whole group $N = 4871$	2 to < 6 years $N = 742$	6 to < 12 years $N = 4129$	Whole group $N = 861$	6 to <12 years $N = 352$	12 to 15 years $N = 509$
Age (mean, SD)	8.21 (1.82)	5.17 (0.64)	8.76 (1.37)	12.13 (1.75)	10.27 (1.01)	13.42 (0.64)
Sex (*N*, %)						
Boys	2431 (49.9)	370 (49.9)	2061 (49.9)	406 (47.2)	161 (45.7)	245 (48.1)
Girls	2440 (50.1)	372 (50.1)	2068 (50.1)	455 (52.8)	191 (54.3)	264 (51.9)
Puberty status (*N*, %)[a]						
Pre or early pubertal	/	/	/	447 (51.9)	297 (84.4)	150 (29.5)
Pubertal	/	/	/	414 (48.1)	55 (15.6)	359 (70.5)
SES (*N*, %)						
Low	436 (9.0)	54 (7.3)	382 (9.3)	54 (6.3)	23 (6.5)	31 (6.1)
Medium	2649 (54.4)	364 (49.1)	2285 (55.3)	434 (50.4)	188 (53.4)	246 (48.3)
High	1786 (36.7)	324 (43.7)	1462 (35.4)	373 (43.3)	141 (40.1)	232 (45.6)
Country (*N*, %)[b]						
Belgium	325 (6.7)	42 (5.7)	283 (6.9)	/	/	/
Cyprus	141 (2.9)	52 (7.0)	89 (2.2)	8 (0.9)	5 (1.4)	3 (0.6)
Estonia	652 (13.4)	8 (1.1)	644 (15.6)	155 (18.0)	0 (0)	155 (30.5)
Germany	779 (16.0)	122 (16.4)	657 (15.9)	259 (30.1)	142 (40.3)	117 (23.0)
Hungary	677 (13.9)	140 (18.9)	537 (13.0)	/	/	/
Italy	1208 (24.8)	186 (25.1)	1022 (24.8)	251 (29.2)	106 (30.1)	145 (28.5)
Spain	771 (15.8)	138 (18.6)	633 (15.3)	188 (21.8)	99 (28.1)	89 (17.5)
Sweden	318 (6.5)	54 (7.3)	264 (6.4)	/	/	/

[a] Pubertal status was not available at baseline survey and indicated with "/"

[b] Countries which did not participate in quantitative ultrasound module were indicated with "/"

At baseline, the average daily nocturnal sleep duration was 9.89 h; the median value of reported daily daytime napping was 60 min. There were 11.2% participants defined as short sleep duration and 6.0% participants defined as long sleep duration. Overall, 44.6% of the participants were defined as poor sleep quality. At follow-up, the average daily nocturnal sleep duration was 9.10 h; the median value of reported daily daytime napping was 31.4 min. The proportions of short and long sleep duration were 16.7% and 8.8%, respectively; 58.8% of participants were reported having poor sleep quality. Furthermore, the average bone stiffness index was 81.91 units at baseline and 91.22 units at follow-up. More details regarding exposures, outcomes, and covariates among pre-school children, primary school children, and adolescents in each survey were shown in Table 2.

Associations between nocturnal sleep, daytime napping, sleep quality, and SI percentiles

In the whole group, no cross-sectional associations between nocturnal sleep duration z-scores, sleep quality, and SI percentiles were observed. Daytime napping duration was positively associated with SI percentiles ($\beta = 0.78$, 95%CI: 0.43, 1.14, $p < 0.001$), and this association was independent of negative effect of screen time and positive effect of sports club on SI percentiles as shown in Online Resource (Table S2). After stratifying by total sleep duration categories, we found that nocturnal sleep duration z-scores were positively associated with SI percentiles in participants with adequate sleep duration ($\beta = 1.81$, 95%CI: 0.55, 3.07, $p = 0.005$), and effect size was even larger, however not statistically significant, in participants with long sleep duration ($\beta = 3.71$, 95%CI: − 2.28, 9.70, $p = 0.22$). Nevertheless, the positive association between daytime napping and SI percentiles was statistically significant only in participants with adequate sleep duration ($\beta = 1.19$, 95%CI: 0.76, 1.61, $p < 0.001$), and was more pronounced but not statistically significant in participants with short sleep duration ($\beta = 1.27$, 95%CI: − 1.14, 3.69, $p = 0.30$) (Table 3).

After 4 years, we found that only follow-up daytime napping was positively associated with follow-up SI percentiles ($\beta = 0.84$, 95%CI: 0.14, 1.53, $p = 0.02$), and it was not influenced by the positive effect of sports club as shown in Online Resource (Table S3), but only existed in participants with short sleep duration after stratification ($\beta = 2.42$, 95%CI: 0.98, 3.85, $p = 0.001$). Even though there were no statistically significant associations between sleep quality and SI percentiles in all groups after 4 years, we observed that participants with poor sleep

Table 2 Baseline and follow-up characteristics of exposures, outcomes, and covariates

	Baseline			Follow-up		
	Whole group *N* = 4871	2 to < 6 years *N* = 742	6 to < 12 years *N* = 4129	Whole group *N* = 861	6 to < 12 years *N* = 352	12 to 15 years *N* = 509
Sleep duration						
Nocturnal sleep duration (hours/day), (mean, SD)	9.89 (0.80)	10.28 (0.85)	9.82 (0.77)	9.10 (1.01)	9.62 (0.71)	8.75 (1.03)
Nocturnal sleep duration z-scores, (mean, SD)	0.33 (0.97)	0.48 (0.96)	0.31 (0.97)	0.09 (0.99)	0.26 (0.92)	− 0.03 (1.02)
Had daytime napping (*n*, %)	522 (10.7)	237 (31.9)	285 (6.9)	119 (13.8)	25 (7.1)	94 (18.5)
Daytime napping duration (10 min/day), (median interquartile range)	6.00 (2.57–9.00)	8.14 (4.29–10.71)	4.29 (2.57–6.43)	3.14 (1.71–7.29)	2.29 (1.71–7.29)	3.82 (1.71–7.29)
Total sleep recommendation (*n*, %)						
Short	545 (11.2)	126 (17.0)	419 (10.1)	144 (16.7)	54 (15.3)	90 (17.7)
Adequate	4032 (82.8)	610 (82.2)	3422 (82.9)	641 (74.5)	289 (82.1)	352 (69.2)
Long	294 (6.0)	6 (0.8)	288 (7.0)	76 (8.8)	9 (2.6)	67 (13.2)
Sleep quality (*n*, %)						
Had an unregularly bedtime routine	1144 (23.5)	171 (23.1)	973 (23.6)	239 (27.8)	48 (13.6)	191 (37.5)
Had difficulty to fall asleep	427 (8.8)	55 (7.4)	372 (9.0)	148 (17.2)	39 (11.1)	109 (21.4)
Had trouble getting up	1117 (22.9)	146 (19.7)	971 (23.5)	359 (41.7)	101 (28.7)	258 (50.7)
Overall poor sleep quality	2172 (44.6)	304 (41.0)	1868 (45.2)	506 (58.8)	147 (41.8)	359 (70.5)
Quantitative ultrasound						
Bone stiffness index, (mean, SD)	81.91 (13.19)	81.06 (16.79)	82.06 (12.43)	91.22 (16.38)	82.82 (12.78)	97.03 (16.09)
Bone stiffness index percentiles, (mean, SD)	46.83 (28.34)	51.62 (30.59)	45.97 (27.84)	53.89 (28.98)	42.47 (28.67)	61.79 (26.48)
Anthropometric measures						
Height (cm), (mean, SD)	131.19 (12.28)	112.78 (6.52)	134.50 (9.92)	154.23 (12.06)	143.91 (8.84)	161.37 (8.21)
Weight (kg), (mean, SD)	30.80 (9.79)	20.53 (4.10)	32.64 (9.36)	48.44 (13.90)	39.32 (10.89)	54.75 (12.16)
Body mass index, (mean, SD)	17.50 (3.30)	16.03 (2.16)	17.77 (3.39)	20.05 (4.00)	18.77 (3.80)	20.93 (3.90)
Body mass index z-scores, (mean, SD)	0.52 (1.21)	0.26 (1.30)	0.56 (1.18)	0.62 (1.08)	0.61 (1.13)	0.62 (1.05)
Health behaviors						
Screen time (hours/week), (mean, SD)	13.90(7.69)	10.88 (6.46)	14.44 (7.77)	16.41 (10.33)	12.49 (6.81)	19.12 (11.43)
Sports club (hours/week), (mean, SD)	1.88 (2.14)	0.75 (1.25)	2.08 (2.20)	2.72 (2.81)	2.43 (2.24)	2.92 (3.13)

[a] Calculated only for participants who reported had daytime napping

quality tended to have lower SI percentiles compared to their counterparts in short ($\beta = -10.17$, 95%CI: − 21.94, 1.61, $p = 0.09$) and long sleep duration group ($\beta = -4.99$, 95%CI: − 22.75, 12.78, $p = 0.56$), respectively (Table 4). The combined effect size was − 11.61 (95%CI: − 21.09, − 2.12, $p = 0.02$) if we merged short and long sleep duration into one group with participants who did not fulfill the sleep recommendation.

Associations between meeting NSF sleep recommendation and SI percentiles

At baseline, there was no statistically significant difference on SI percentile between children who fulfilled the sleep recommendation and who did not. However, extreme total sleep duration at baseline predicted lower SI percentiles after 4 years compared to their counterparts when participants simultaneously had poor sleep quality at baseline ($\beta = -8.09$, 95%CI: − 13.39, − 2.79, $p = 0.003$) (Table 5). As shown in Online Resource (Table S4), four interaction terms in the cross-sectional model and eight in the longitudinal model were separately introduced to the main effects in the whole group, one of which was statistically significant, i.e., the interaction between extreme total sleep duration at baseline and average screen time ($\beta = -0.52$, 95%CI: − 1.02, − 0.03, $p = 0.04$). Specifically, screen time has a stronger detrimental effect on SI percentile in children with extreme total sleep duration.

Discussion

We observed a positive cross-sectional association between nocturnal sleep duration and SI only in participants with

Table 3 Cross-sectional associations between sleep characteristics and bone stiffness index percentiles in 2009/10, stratified by total sleep duration[a]

	Whole group		Short sleep duration		Adequate sleep duration		Long sleep duration	
	(*N* = 4871)		(*N* = 545)		(*N* = 4032)		(*N* = 294)	
	β (95%CI)	*p* value	*β* (95%CI)	*p* value	*β* (95%CI)	*p* value	*β* (95%CI)	*p* value
Nocturnal sleep duration z-scores	0.26 (− 0.63, 1.14)	0.57	− 1.25 (− 4.82, 2.31)	0.49	1.81 (0.55, 3.07)	0.005	3.71 (− 2.28, 9.70)	0.22
Daytime napping	0.78 (0.43, 1.14)	*p* < 0.001	1.27 (− 1.14, 3.69)	0.30	1.19 (0.76, 1.61)	*p* < 0.001	0.36 (− 1.05, 1.78)	0.61
Sleep quality								
Poor vs. good (reference)	− 0.44 (− 2.09, 1.22)	0.61	0.87 (− 4.12, 5.86)	0.73	− 0.88 (− 2.70, 0.93)	0.34	1.42 (− 5.84, 8.69)	0.70

All models were adjusted for sex, age, family socioeconomic status, screen time, time spent at sports clubs, BMI z-scores, and daylight duration, with a random effect for country

[a] Based on the sleep recommendation from the National Sleep Foundation

adequate sleep duration. In general, the positive association between daytime napping and SI was more pronounced in participants with short and adequate sleep duration. Moreover, extreme sleep duration at baseline predicted lower SI over the 4 years of follow-up in participants with poor sleep quality. Besides, a negative association of SB and a positive association of PA with SI were observed, which are consistent with previous IDEFICS/I.Family findings [27, 28]. However, these known factors influencing bone development did not modify the associations between sleep exposures and SI in

Table 4 Longitudinal associations between sleep characteristics and bone stiffness index percentiles in 2013/14, stratified by total sleep duration [a]

	Whole group		Short sleep duration		Adequate sleep duration		Long sleep duration	
	(*N* = 861)		(*N* = 101)		(*N* = 722)		(*N* = 38)	
	β (95%-CI)	*p* v-al-ue	*β* (95%C-I)	*p* v-al-ue	*β* (95%-CI)	*p* v-al-ue	*β* (95%C-I)	*p* v-al-ue
Baseline nocturnal sleep duration z-scores	− 0.43 (− 2.30, 1.44)	0.65	− 0.17 (− 10.53, 10.18)	0.97	− 1.31 (− 3.85, 1.23)	0.31	− 9.52 (− 24.27, 5.23)	0.19
Baseline daytime napping	− 1.06 (− 2.05, − 0.07)	0.04 [c]	− 4.80 (− 14.78, 5.18)	0.34	− 1.03 (− 2.48, 0.42)	0.16	− 1.73 (− 5.05, 1.58)	0.29
Follow-up nocturnal sleep duration z-scores	− 0.73 (− 2.35, 0.89)	0.38	1.11 (− 4.65, 6.87)	0.70	− 0.53 (− 2.30, 1.24)	0.56	− 0.36 (− 6.06, 5.34)	0.90
Follow-up daytime napping	0.84 (0.14, 1.53)	0.02	2.42 (0.98, 3.85)	0.001	0.37 (− 0.47, 1.21)	0.39	0.52 (− 3.13, 4.18)	0.77
Sleep quality[b]								
Poor vs. good (reference)	− 1.29 (− 4.98, 2.40)	0.49	− 10.17 (− 21.94, 1.61)	0.09	0.12 (− 3.85, 4.08)	0.95	− 4.99 (− 22.75, 12.78)	0.56

All models were adjusted for bone stiffness index percentiles at baseline, sex, age, family socioeconomic status, pubertal status, BMI z-scores, and daylight duration at follow-up as well as average screen time and time spent at sports clubs at both surveys

[a] Based on the sleep recommendation from the National Sleep Foundation

[b] Good sleep quality was defined as fulfilling at both baseline and follow-up

[c] $p \geq 0.05$ after adjustment for multiple testing according to Holm's sequential Bonferroni procedure

Table 5 Cross-sectional and longitudinal associations between meeting sleep guidelines [a] and bone stiffness index percentiles, stratified by sleep quality

	Whole group		Poor sleep quality		Good sleep quality	
	β (95%CI)	p value	β (95%CI)	p value	β (95%CI)	p value
Cross-sectional models	$N = 4871$		$N = 2172$		$N = 2699$	
Short or long vs. adequate (reference)	− 0.49 (− 2.46, 1.47)	0.62	0.82 (− 1.91, 3.55)	0.56	− 1.89 (− 4.73, 0.94)	0.19
Longitudinal models	$N = 861$		$N = 405$		$N = 456$	
Baseline short or long vs. adequate (reference)	− 3.45 (− 7.61, 0.70)	0.10	− 8.09 (− 13.39, − 2.79)	0.003	1.28 (− 5.17, 7.73)	0.70
Follow-up short or long vs. adequate (reference)	− 0.55 (− 4.09, 2.99)	0.76	0.49 (− 4.26, 5.25)	0.84	− 3.08 (− 8.31, 2.14)	0.25

All cross-sectional models were adjusted for sex, age, family socioeconomic status, screen time, time spent at sports clubs, BMI z-scores, and daylight duration, with a random effect for country

All longitudinal models were adjusted for bone stiffness index percentiles at baseline, sex, age, family socioeconomic status, pubertal status, BMI z-scores, and daylight duration at follow-up as well as average screen time and time spent at sports clubs at both surveys

All whole models were additionally adjusted for sleep quality

[a] Based on the sleep recommendation from the National Sleep Foundation

the present study. In addition, we found extreme sleep duration had an interactive effect on the association between screen time and SI, suggesting that sleep duration should also be taken into consideration in further studies on behavioral risk factors for bone health.

To our best knowledge, there were only three studies investigating the associations between sleep duration and bone density measured by DXA among children and adolescents and reporting conflicting results: a cross-sectional study among 4–12 year-old children found positive associations of long nocturnal sleep duration and daytime napping with total BMC [15]. Another study conducted among 6–18 year-old Japanese students reported that habitual napping rather than nocturnal sleep duration was positively associated with BMD at the distal forearm of the non-dominant side [16]. The last, a longitudinal study using compositional time of 24 h day, reported that total sleep duration was not related to BMC and BMD [17]. QUS, as a fast, radiation-free and cost-effective technique, not only offers better accessibility for large-scale epidemiological studies particularly involving healthy children and adolescents but also provides some information on the structural and geometric properties of the bone in addition to bone density measured by DXA. The predictive values of both BUA and SOS for osteoporotic fracture have been supported by a systematic review on prospective studies, albeit decreased with time [29]. Some researchers propose to use more sophisticated QUS indices, e.g., SI. Compared with using BUA and SOS alone, SI showed more sensitivity for subjects with low BMD [30] and better long-term precision to monitor the treatment effect of therapies [31, 32]. However, there are still some concerns on the QUS methodology because of the poor knowledge on bone properties reflected by QUS parameters, the influence of body size, and technological diversity among QUS devices and indices [24]. Therefore, the comparison of our study with previous research is limited and our results should be carefully interpreted.

In the present study, we found that longer nocturnal sleep duration would not be beneficial to bone health if children had inadequate total sleep duration. In contrast, more daytime napping could make up for the deleterious effect of inadequate total sleep duration on bone. However, our results were in contrast to the findings from previous studies in adults, which suggested that daytime napping duration was a risk factor of lower BMD [33, 34]. According to previous systematic reviews regarding other health effects of daytime napping, the risks of detrimental outcomes (i.e., cardiovascular disease, and type diabetes and metabolic syndrome) showed a J-curve dose-response with no effect [35] or decreases [36] up to about 40 min/day, and then followed by sharp increases. Moreover, a study conducted in American high school students suggested that only specific time period of taking naps (e.g., after 2 pm) was related to increasing inflammatory factors [37]. However, only about 6% of the children reported their daytime napping was more than 40 min/day in our sample. These results indicated that acceptable duration of daytime napping may be good for bone health in children and adolescents who did not have long sleep duration. However, the optimal threshold and timing of napping are still needed to be confirmed.

Healthy sleep consists of adequate duration and good quality. Although individuals with extreme sleep duration (short or long) tend to have poor sleep quality and vice versa, it is still possible that some individuals naturally need less or more sleep compared to others, only if good sleep quality is maintained under individually preferred sleep duration [38]. On the other hand, individuals with poor sleep quality also may partly compensate by keeping adequate sleep duration. Nonetheless, relatively few studies simultaneously examined the effects of extreme sleep duration and sleep quality. In order to preclude the interplay of sleep duration and quality in the present study,

we explored the associations stratified by sleep duration and sleep quality, suggested that the associations of sleep duration and quality with bone health may be interrelated. These findings were supported by previous studies in adults. For example, Zeng et al. [39] reported that long sleep duration was a risk factor for poor quality of life in patients with type 2 diabetes mellitus who reported poor sleep quality. In contrast, Chen et al. [33] found the association between sleep duration and osteoporosis was most pronounced in postmenopausal women reported good sleep quality. Given the limited and conflicting evidence, the interactive effect between sleep duration and sleep quality on bone is still unknown.

Experimental sleep deprivation studies have demonstrated that sleep restriction may lead to increase of bone resorption markers and decrease of bone formation markers, thereby impacting the bone mass accrual [40, 41]. Compared to the detrimental effects of short sleep duration, evidence of long sleep duration is still lack among youth, and the underlying mechanism for adverse effects of long sleep duration on health is rarely investigated because of the difficulty to conduct experimental studies. However, a recent systematic review suggested that long sleep duration, but not short sleep duration, was associated with increased inflammatory factors [9]. Moreover, long sleep duration may decrease daily exercises and thus results in less bone stimulation from mechanical loading [42]. Furthermore, extreme sleep duration and poor quality always paralleled with the poor sleep efficiency and interruption of circadian rhythm [43]. Growth hormone (GH) levels are increased during sleep period. GH and insulin-like growth factor 1 (IGF-1) have been demonstrated to stimulate osteoblasts in the bone, and GH also effects musculoskeletal system directly as well as through mediating IGF-1 levels [44]. In addition, the circadian rhythm is related to hypothalamic-pituitary-adrenal axis which is typically relates to the release of cortisol. High level of cortisol can exert detrimental effects on the musculoskeletal system directly and by inhibiting GH and IGF-1 [44, 45]. Considering that the increases of extreme sleep duration and poor sleep quality have raised a concern from early life span, more perspective studies are needed to establish causality as well as the underlying mechanisms on bone health.

The main strength of the present study is that we fill the research gap regarding the associations and interplays of nocturnal sleep duration, daytime napping, and sleep quality with SI. Moreover, the prospective study design, the standardized measures providing harmonized data across eight European countries, and the large sample size covering children from 2 to 15 years old strengthen our findings. The deep phenotyping also allows consideration of a number of important confounders, i.e., family SES, pubertal status, BMI, PA, and SB. However, some limitations should be acknowledged. First, the QUS measurement was only available in a subgroup with decreasing sample size over the 4 years of follow-up, which may cause selective bias in data interpretation. Second, sleep duration was reported by parents or adolescents, and sleep quality was estimated from only three sleep characteristics. Finally, we did not have information regarding some possible confounders, e.g., weight-bearing exercises and calcium intake. Instead, we used proxy indicators of reported types of sport in sports club (moderate or high mechanical loads vs. no or low mechanical loads) and milk and dairy products consumption (frequency/week). However, no modifying effects were observed in the exploratory analysis stage; hence, we did not include them in the final models in order to maintain the sample size.

In conclusion, we observed that for every 10 min/day increase in daytime napping was associated with approximately 1 unit increase in SI percentiles, and it was even more beneficial for individuals who had short sleep duration according to NSF sleep recommendation. Furthermore, the associations of sleep duration and sleep quality with calcaneus SI may partly depend on each other. We suggest that children and adolescents should follow the NSF sleep recommendation in order to maximize bone strength during growth, especially for those who had poor sleep quality measured by no regular bedtime routine, had trouble getting up in the morning and difficulty falling asleep.

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Data availability The datasets of the present study are not publicly available since it is highly sensitive data collected in young children. Interested researchers can contact the IDEFICS and I.Family consortia (http://www.ideficsstudy.eu/Idefics/ and https://www.ifamilystudy.eu/) for possible data access.

Compliance with ethical standards

Conflict of interest None.

Ethics approval Ethical approval was obtained from the ethics committees for all participating centers in each country: Ethics Committee, University Hospital, Gent, Belgium; Cyprus National Bioethics Committee, Nicosia, Cyprus; Tallinn Medical Research Ethics Committee, Tallinn, Estonia; Ethics Committee of the University of Bremen, Bremen, Germany; Egészségügyi Tudományos Tanács, Pécs, Hungary; Azienda Sanitaria Locale Avellino Comitato Etico, Avellino, Italy; Regionala Etikprövningsnämnden i Göteborg, Gothenburg, Sweden; Comité Ético de Investigación Clínica de Aragón, Zaragoza,

Spain. All the examinations were performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki.

Consent to participate All parents signed informed consent, in addition, children aged 12 years and older gave simplified written consent and children younger than 12 years gave oral consent prior to the examinations in addition to the signed parental consent.

Consent for publication Not applicable

Code availability All the statistical analyses were carried out with the Statistical Analysis System (SAS) software package (Version 9.4; SAS Institute, Cary, NC).

References

1. Matricciani L, Olds T, Petkov J (2012) In search of lost sleep: secular trends in the sleep time of school-aged children and adolescents. Sleep Med Rev 16(3):203–211. https://doi.org/10.1016/j.smrv.2011.03.005
2. Crowley SJ, Van Reen E, LeBourgeois MK, Acebo C, Tarokh L, Seifer R, Barker DH, Carskadon MA (2014) A longitudinal assessment of sleep timing, circadian phase, and phase angle of entrainment across human adolescence. PLoS One 9(11):e112199. https://doi.org/10.1371/journal.pone.0112199
3. Fricke-Oerkermann L, Pluck J, Schredl M, Heinz K, Mitschke A, Wiater A, Lehmkuhl G (2007) Prevalence and course of sleep problems in childhood. Sleep 30(10):1371–1377. https://doi.org/10.1093/sleep/30.10.1371
4. Hysing M, Pallesen S, Stormark KM, Lundervold AJ, Sivertsen B (2013) Sleep patterns and insomnia among adolescents: a population-based study. J Sleep Res 22(5):549–556. https://doi.org/10.1111/jsr.12055
5. Hausler N, Marques-Vidal P, Haba-Rubio J, Heinzer R (2019) Does sleep predict next-day napping or does napping influence same-day nocturnal sleep? Results of a population-based ecological momentary assessment study. Sleep Med 61:31–36. https://doi.org/10.1016/j.sleep.2019.04.014
6. Paruthi S, Brooks LJ, D'Ambrosio C, Hall WA, Kotagal S, Lloyd RM, Malow BA, Maski K, Nichols C, Quan SF, Rosen CL, Troester MM, Wise MS (2016) Recommended amount of sleep for pediatric populations: a consensus statement of the american Academy of Sleep Medicine. J Clin Sleep Med 12(6):785–786. https://doi.org/10.5664/jcsm.5866
7. Potter GD, Skene DJ, Arendt J, Cade JE, Grant PJ, Hardie LJ (2016) Circadian rhythm and sleep disruption: causes, metabolic consequences, and countermeasures. Endocr Rev 37(6):584–608. https://doi.org/10.1210/er.2016-1083
8. Kuriyama N, Inaba M, Ozaki E, Yoneda Y, Matsui D, Hashiguchi K, Koyama T, Iwai K, Watanabe I, Tanaka R, Omichi C, Mizuno S, Kurokawa M, Horii M, Niwa F, Iwasa K, Yamada S, Watanabe Y (2017) Association between loss of bone mass due to short sleep and leptin-sympathetic nervous system activity. Arch Gerontol Geriatr 70:201–208. https://doi.org/10.1016/j.archger.2017.02.005
9. Irwin MR, Olmstead R, Carroll JE (2016) Sleep disturbance, sleep duration, and inflammation: a systematic review and meta-analysis of cohort studies and experimental sleep deprivation. Biol Psychiatry 80(1):40–52. https://doi.org/10.1016/j.biopsych.2015.05.014
10. Krietsch KN, Chardon ML, Beebe DW, Janicke DM (2019) Sleep and weight-related factors in youth: a systematic review of recent studies. Sleep Med Rev 46:87–96. https://doi.org/10.1016/j.smrv.2019.04.010
11. Wang D, Ruan W, Peng Y, Li W (2018) Sleep duration and the risk of osteoporosis among middle-aged and elderly adults: a dose-response meta-analysis. Osteoporos Int 29(8):1689–1695. https://doi.org/10.1007/s00198-018-4487-8
12. Lucassen EA, de Mutsert R, le Cessie S, Appelman-Dijkstra NM, Rosendaal FR, van Heemst D, den Heijer M, Biermasz NR (2017) Poor sleep quality and later sleep timing are risk factors for osteopenia and sarcopenia in middle-aged men and women: the NEO study. PLoS One 12(5):e0176685. https://doi.org/10.1371/journal.pone.0176685
13. Sasaki N, Fujiwara S, Yamashita H, Ozono R, Teramen K, Kihara Y (2016) Impact of sleep on osteoporosis: sleep quality is associated with bone stiffness index. Sleep Med 25:73–77. https://doi.org/10.1016/j.sleep.2016.06.029
14. Weaver CM, Gordon CM, Janz KF, Kalkwarf HJ, Lappe JM, Lewis R, O'Karma M, Wallace TC, Zemel BS (2016) The National Osteoporosis Foundation's position statement on peak bone mass development and lifestyle factors: a systematic review and implementation recommendations. Osteoporos Int 27(4):1281–1386. https://doi.org/10.1007/s00198-015-3440-3
15. Casazza K, Hanks LJ, Fernandez JR (2011) Shorter sleep may be a risk factor for impaired bone mass accrual in childhood. J Clin Densitom 14(4):453–457. https://doi.org/10.1016/j.jocd.2011.06.005
16. Nakagi Y, Ito T, Hirooka K, Sugioka Y, Endo H, Saijo Y, Imai H, Takeda H, Kayama F, Sasaki S, Yoshida T (2010) Association between lifestyle habits and bone mineral density in Japanese juveniles. Environ Health Prev Med 15(4):222–228. https://doi.org/10.1007/s12199-009-0131-8
17. Taylor RW, Haszard JJ, Meredith-Jones KA, Galland BC, Heath AM, Lawrence J, Gray AR, Sayers R, Hanna M, Taylor BJ (2018) 24-h movement behaviors from infancy to preschool: cross-sectional and longitudinal relationships with body composition and bone health. Int J Behav Nutr Phys Act 15(1):118. https://doi.org/10.1186/s12966-018-0753-6
18. Ahrens W, Siani A, Adan R, De Henauw S, Eiben G, Gwozdz W, Hebestreit A, Hunsberger M, Kaprio J, Krogh V, Lissner L, Molnar D, Moreno LA, Page A, Pico C, Reisch L, Smith RM, Tornaritis M, Veidebaum T, Williams G, Pohlabeln H, Pigeot I, consortium IF (2017) Cohort profile: the transition from childhood to adolescence in European children-how I.Family extends the IDEFICS cohort. Int J Epidemiol 46(5):1394–1395j. https://doi.org/10.1093/ije/dyw317
19. Hirshkowitz M, Whiton K, Albert SM, Alessi C, Bruni O, DonCarlos L, Hazen N, Herman J, Adams Hillard PJ, Katz ES, Kheirandish-Gozal L, Neubauer DN, O'Donnell AE, Ohayon M, Peever J, Rawding R, Sachdeva RC, Setters B, Vitiello MV, Ware JC (2015) National Sleep Foundation's updated sleep duration recommendations: final report. Sleep Health 1(4):233–243. https://doi.org/10.1016/j.sleh.2015.10.004

20. Magee CA, Robinson L, Keane C (2017) Sleep quality subtypes predict health-related quality of life in children. Sleep Med 35:67–73. https://doi.org/10.1016/j.sleep.2017.04.007
21. UNESCO Institute for Statistics (2012) International standard classification of education: ISCED 2011. UNESCO Institute for Statistics, Montreal
22. Saraff V, Shaw N (2016) Sunshine and vitamin D. Arch Dis Child 101(2):190–192. https://doi.org/10.1136/archdischild-2014-307214
23. Herrmann D, Intemann T, Lauria F, Marild S, Molnar D, Moreno LA, Sioen I, Tornaritis M, Veidebaum T, Pigeot I, Ahrens W, consortium I (2014) Reference values of bone stiffness index and C-terminal telopeptide in healthy European children. Int J Obes 38(Suppl 2):S76–S85. https://doi.org/10.1038/ijo.2014.138
24. Baroncelli GI (2008) Quantitative ultrasound methods to assess bone mineral status in children: technical characteristics, performance, and clinical application. Pediatr Res 63(3):220–228. https://doi.org/10.1203/PDR.0b013e318163a286
25. Cole TJ, Lobstein T (2012) Extended international (IOTF) body mass index cut-offs for thinness, overweight and obesity. Pediatr Obes 7(4):284–294. https://doi.org/10.1111/j.2047-6310.2012.00064.x
26. Holm S (1979) A simple sequentially rejective multiple test procedure. Scand J Stat 6:65–70
27. Cheng L, Pohlabeln H, Ahrens W, Lauria F, Veidebaum T, Chadjigeorgiou C, Molnár D, Eiben G, Michels N, Moreno LA, Page AS, Pitsiladis Y, Hebestreit A (2020) Cross-sectional and longitudinal associations between physical activity, sedentary behaviour and bone stiffness index across weight status in European children and adolescents. Int J Behav Nutr Phys Act 17(1):54. https://doi.org/10.1186/s12966-020-00956-1
28. Herrmann D, Buck C, Sioen I, Kouride Y, Marild S, Molnar D, Mouratidou T, Pitsiladis Y, Russo P, Veidebaum T, Ahrens W, consortium I (2015) Impact of physical activity, sedentary behaviour and muscle strength on bone stiffness in 2-10-year-old children-cross-sectional results from the IDEFICS study. Int J Behav Nutr Phys Act 12:112. https://doi.org/10.1186/s12966-015-0273-6
29. McCloskey EV, Kanis JA, Odén A, Harvey NC, Bauer D, González-Macias J, Hans D, Kaptoge S, Krieg MA, Kwok T, Marin F, Moayyeri A, Orwoll E, Gluër C, Johansson H (2015) Predictive ability of heel quantitative ultrasound for incident fractures: an individual-level meta-analysis. Osteoporos Int 26(7):1979–1987. https://doi.org/10.1007/s00198-015-3072-7
30. Guglielmi G, de Terlizzi F (2009) Quantitative ultrasond in the assessment of osteoporosis. Eur J Radiol 71(3):425–431. https://doi.org/10.1016/j.ejrad.2008.04.060
31. Gonnelli S, Cepollaro C, Montagnani A, Martini S, Gennari L, Mangeri M, Gennari C (2002) Heel ultrasonography in monitoring alendronate therapy: a four-year longitudinal study. Osteoporos Int 13(5):415–421. https://doi.org/10.1007/s001980200048
32. Sahota O, San P, Cawte SA, Pearson D, Hosking DJ (2000) A comparison of the longitudinal changes in quantitative ultrasound with dual-energy X-ray absorptiometry: the four-year effects of hormone replacement therapy. Osteoporos Int 11(1):52–58. https://doi.org/10.1007/s001980050006
33. Chen G, Chen L, Wen J, Yao J, Li L, Lin L, Tang K, Huang H, Liang J, Lin W, Chen H, Li M, Gong X, Peng S, Lu J, Bi Y, Ning G (2014) Associations between sleep duration, daytime nap duration, and osteoporosis vary by sex, menopause, and sleep quality. J Clin Endocrinol Metab 99(8):2869–2877. https://doi.org/10.1210/jc.2013-3629
34. Saetung S, Reutrakul S, Chailurkit LO, Rajatanavin R, Ongphiphadhanakul B, Nimitphong H (2018) The association between daytime napping characteristics and bone mineral density in elderly thai women without osteoporosis. Sci Rep 8(1):10016. https://doi.org/10.1038/s41598-018-28260-w
35. Yamada T, Shojima N, Yamauchi T, Kadowaki T (2016) J-curve relation between daytime nap duration and type 2 diabetes or metabolic syndrome: a dose-response meta-analysis. Sci Rep 6:38075. https://doi.org/10.1038/srep38075
36. Yamada T, Hara K, Shojima N, Yamauchi T, Kadowaki T (2015) Daytime napping and the risk of cardiovascular disease and all-cause mortality: a prospective study and dose-response meta-analysis. Sleep 38(12):1945–1953. https://doi.org/10.5665/sleep.5246
37. Jakubowski KP, Hall MH, Marsland AL, Matthews KA (2016) Is daytime napping associated with inflammation in adolescents? Health Psychol 35(12):1298–1306. https://doi.org/10.1037/hea0000369
38. Chaput JP, Dutil C, Sampasa-Kanyinga H (2018) Sleeping hours: what is the ideal number and how does age impact this? Nat Sci Sleep 10:421–430. https://doi.org/10.2147/NSS.S163071
39. Zeng Y, Wu J, Yin J, Chen J, Yang S, Fang Y (2018) Association of the combination of sleep duration and sleep quality with quality of life in type 2 diabetes patients. Qual Life Res 27(12):3123–3130. https://doi.org/10.1007/s11136-018-1942-0
40. Staab JS, Smith TJ, Wilson M, Montain SJ, Gaffney-Stomberg E (2019) Bone turnover is altered during 72 h of sleep restriction: a controlled laboratory study. Endocrine 65(1):192–199. https://doi.org/10.1007/s12020-019-01937-6
41. Swanson CM, Shea SA, Wolfe P, Cain SW, Munch M, Vujovic N, Czeisler CA, Buxton OM, Orwoll ES (2017) Bone turnover markers after sleep restriction and circadian disruption: a mechanism for sleep-related bone loss in humans. J Clin Endocrinol Metab 102(10):3722–3730. https://doi.org/10.1210/jc.2017-01147
42. Master L, Nye RT, Lee S, Nahmod NG, Mariani S, Hale L, Buxton OM (2019) Bidirectional, daily temporal associations between sleep and physical activity in adolescents. Sci Rep 9(1):7732. https://doi.org/10.1038/s41598-019-44059-9
43. Buysse DJ (2014) Sleep health: can we define it? Does it matter? Sleep 37(1):9–17. https://doi.org/10.5665/sleep.3298
44. Perrini S, Laviola L, Carreira MC, Cignarelli A, Natalicchio A, Giorgino F (2010) The GH/IGF1 axis and signaling pathways in the muscle and bone: mechanisms underlying age-related skeletal muscle wasting and osteoporosis. J Endocrinol 205(3):201–210. https://doi.org/10.1677/joe-09-0431
45. Leproult R, Copinschi G, Buxton O, Van Cauter E (1997) Sleep loss results in an elevation of cortisol levels the next evening. Sleep 20(10):865–870

Paper 4

Associations between serum 25-hydroxyvitamin D, bone turnover markers and bone stiffness in European children and adolescents

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Short running head: vitamin D and bone health indicators in children

Abbreviations list:

25(OH)D: 25-hydroxyvitamin D

BMC: bone mineral content

BMD: bone mineral density

DXA: dual-energy X-ray absorptiometry

QUS: quantitative ultrasound

SI: stiffness index

IDEFICS: Identification and Prevention of Dietary and Lifestyle-Induced Health Effects in Children and Infants

PA: physical activity

OC: osteocalcin

PINP: N-terminal propeptide of type I procollagen

ALP: alkaline phosphatase

CTx: C-terminal telopeptides of type I collagen

NTx: N-terminal telopeptides of type I collagen

SB: sedentary behavior

IOM: Institute of Medicine

ISCD: International Society for Clinical Densitometry

$CV_{RMS:}$ root-mean-square coefficient of variation

ROI: region of interest

SOS: speed of sound

BUA: broadband ultrasound attenuation

BMI: body mass index

EFSA: European Food Safety Authority

cpm: counts per minute

MVPA: moderate-to-vigorous physical activity

ISCED: International Standard Classification of Education

UVI: ultraviolet radiation index

SD: standard deviation

CI: confidence interval

PTH: parathyroid hormone

Clinical Trial Registry: Pan-European IDEFICS/I.Family children cohort; ISRCTN62310987; https://doi.org/10.1186/ISRCTN62310987

Data sharing: The datasets generated and analyzed during the present study are not public. However, interested researchers can contact the IDEFICS and I. Family consortia (http://www.ideficsstudy.eu/Idefics/andhttp://www.ifamilystudy.eu/) to discuss the possibilities of data access.

Abstract

Background The relationships between serum 25-hydroxyvitamin D concentration (25(OH)D), bone turnover markers and calcaneal quantitative ultrasound measured stiffness index (SI) in childhood are unclear.

Objective To investigate the associations of 25(OH)D with osteocalcin (OC), C-terminal telopeptides of type I collagen (CTx) and SI, and to explore the interactions of physical activity, calcium intake and weight status with 25(OH)D on bone health indicators.

Design 25(OH)D, OC, CTx and SI were assessed in 2- to 15-year-old European children from examinations in 2007/08 and 2013/14. Time spent in sports clubs (hours/week), screen time duration (hours/week) and dairy products consumption (frequency/week) were reported using questionnaires. Moderate-to-vigorous physical activity (MVPA, min/day) and sedentary time (min/day) were measured using accelerometers. Calcium intake (mg/day) was measured using 24-h dietary recall and dichotomized. Overweight/obesity was defined based on bone mass index. Linear mixed-effects models were used with adjustments for potential confounders, cluster effect of country and repeated measurements.

Results 25(OH)D (β = -7.09), dairy products consumption (β = -1.64) and dichotomized calcium intake (β = -116.70) were inversely associated with CTx. 25(OH)D ≥ 20 ng/ml was a protective factor for SI only in children with MVPA ≥ 60 min/day (β = 12.14). Higher OC was observed in preschool children had MVPA ≥ 60 min/day than who did not. Interaction between 25(OH)D and weight status on CTx was observed in primary school children. Time spent in sports clubs was positively while screen time duration was inversely associated with CTx in adolescents. In the 2013/14 examination, positive associations of time spent in sports clubs (β = 1.28), MVPA (β = 0.20), dairy products consumption (β = 0.15) and calcium intake (β = 0.02) with SI were observed.

Conclusions 25(OH)D was reversely associated with CTx. Future intervention studies on vitamin D and bone health should consider MVPA, weight status and age.

Keywords vitamin D; bone health; calcium intake; physical activity; weight status; child cohort

Introduction

The high prevalence of vitamin D deficiency among European children and adolescents has raised a considerable concern in public health in the past decades (1). Vitamin D, as a pro-hormone, has been related to a wide variety of pathogenesis including infections (2), cardiovascular diseases (3) and mental health (4), etc. The most well-established health effect of vitamin D is its role in the regulation of calcium and phosphate homeostasis, which is important for bone health. Vitamin D can be obtained from dietary intake, but the majority source of vitamin D3 is synthesized from 7-dehydrocholesterol in skin in response to ultraviolet-B radiation, then converted to 25-hydroxyvitamin D (25(OH)D) in the liver and finally converted to the active metabolite 1,25-dihydroxyvitamin D in kidney. In childhood, the most severe clinical consequence of vitamin D deficiency is impaired bone mass acquisition and rickets (5).

Serum 25(OH)D is commonly used as an indicator of the vitamin D status. However, the associations between serum 25(OH)D and indicators of bone health e.g., bone mineral content (BMC) and density (BMD) measured using dual-energy X-ray absorptiometry (DXA) are yet unclear, with reported positive (6), negative (7) or no associations (8) in children. Although some studies used 25(OH)D cut-offs and found that children with higher levels of 25(OH)D had more BMC and BMD (9, 10), the effectiveness of vitamin D supplementation in intervention studies is still inconclusive (11). Furthermore, bone strength not only depends on bone mass and density but also on other structural properties. Quantitative ultrasound (QUS) measured bone stiffness index (SI) as a proxy indicator for bone strength, is estimated based on the speed and attenuation of the ultrasound as it passes through the bone, which are related to both BMD and structural strength (12). In a case-control study embedded in the IDEFICS study (Identification and Prevention of Dietary and Lifestyle-Induced Health Effects in Children and Infants), combined effect of low serum 25(OH)D with low physical activity (PA) was observed on poor SI (13).

Biochemical bone turnover markers provide a dynamic representation of bone metabolism, which also contributes to bone health in addition to bone strength measured by densitometries. The most commonly used markers for bone formation are osteocalcin (OC), N-terminal pro-peptide of type I pro-collagen (PINP) and alkaline phosphatase (ALP), and for bone resorption are C- and N-terminal telopeptides of type I collagen (CTx and NTx) (14). In adults and elderly, the increases of bone turnover markers represent high rate of bone remodeling and resulting in bone loss (15). However, in healthy children and adolescents, bone turnover involved in both modeling and remodeling is relatively rapid due to the high demand of skeletal growth. Even though few longitudinal studies reported positive associations between serum bone turnover markers at baseline and gain in BMC and BMD at follow-up (16, 17), most of evidence supports their inverse associations in children and adolescents (18).

Hence, the present study used bone formation marker serum OC, bone resorption markers serum CTx and QUS-derived SI as indicators of bone health, aimed to investigate the associations of serum 25(OH)D with bone turnover and bone stiffness. The study further aimed to explore the interplays of PA, calcium intake and weight status with 25(OH)D on bone health indicators.

Subjects and Methods

Study design

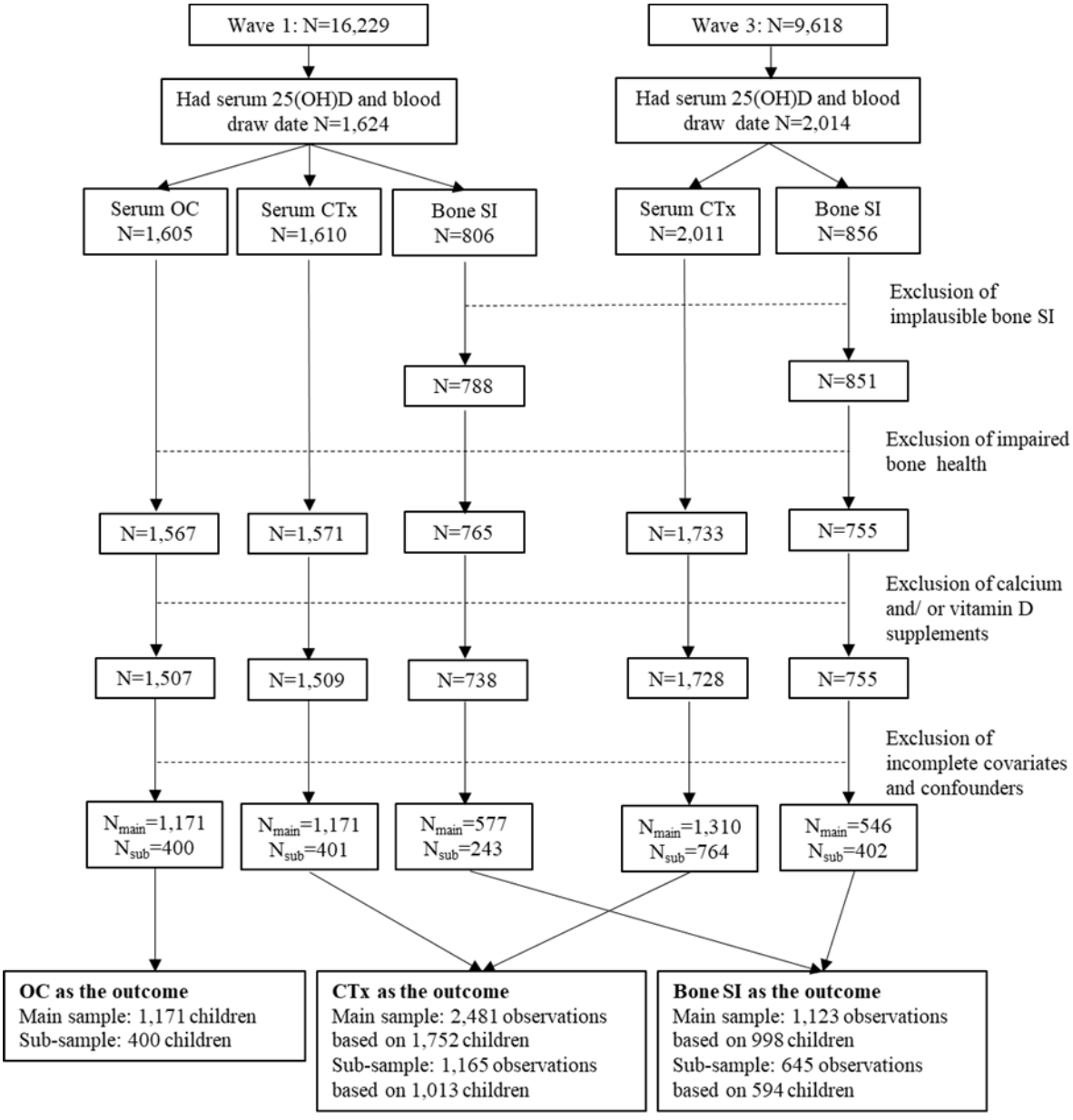
The IDEFICS/I.Family study is a prospective cohort study comprising a large set of examinations including questionnaires, physical examinations, measurements, tests and biological samples, etc. The baseline survey (wave 1) started in 2007/2008 in eight European countries (Sweden, Germany, Hungary, Italy, Cyprus, Spain, Belgium and Estonia) and included 16,229 children aged 2-9.9 years. The second examination (wave 2) was conducted with a follow-up of 11,043 children and 2,543 newly recruited children after 2 years in 2009/2010 (19). The third examination (wave 3) included 7,117 children who had participated in previous

examinations and 2,501 newly recruited children – mainly siblings and aimed to further investigate the determinants of health behaviors among children and their families (20). The study procedures were in accordance with the Helsinki Declaration. All recruitment centers obtained approvals from their local ethics committees. Parents and adolescents aged 12 years and older gave written informed consent, children less than 12 years gave their verbal assent before participating in each examination. All study nurses and examination staff received central or local training; all the examinations and quality control were adhered to the standard operation procedures. The pan-European IDEFICS/I.Family children cohort has been registered at the ISRCTN clinical trials registry (ISRCTN62310987).

Subjects

Laboratory measurements of serum 25(OH)D and blood draw dates were available for 1,624 children aged 2 to 9 years in wave 1 and for 2,014 children aged 7 to 15 years in wave 3. Only children with at least one available bone measurement of serum OC, CTx or calcaneal SI were included in the present study. We further excluded children who (1) had implausible QUS measurements, i.e., absolute difference of SI between the right and left foot exceeded the 97th percentile (41 units); (2) reported an indication of impaired bone health e.g., disease or receiving medical treatments affecting the bone; (3) reported using nutrition supplementations e.g., calcium or vitamin D supplementations and combinations; (4) had incomplete data of covariates and confounders e.g., parental education level. Finally, for each bone health indicator (serum OC, CTx and SI), a main sample with full information of questionnaire-based PA, sedentary behavior (SB) and consumption frequency of dairy products, as well as a sub-sample with full information of accelerometer-based PA, SB and calcium intake from 24-h dietary recall were generated (Figure 1).

Figure 1 Flow chart of children included in the main samples and the sub-samples for bone health indicators of serum OC, CTx and calcaneal SI; the baseline examination in 2007/2008 (wave 1), the third examination in 2013/2014 (wave 3); main samples consist of children with full information on self-reported physical activity, sedentary behavior and consumption frequency of dairy products, sub-samples consist of children had accelerometer data and calcium intake measured using 24-h dietary recall; *25(OH)D* 25-hydroxyvitamin D *OC* osteocalcin *CTx* C-terminal telopeptides of type I collagen *SI* stiffness index



Serum 25(OH)D concentrations and bone turnover markers

Fasting blood samples through venipuncture sampling were collected in the morning and immediately processed to separate serum and plasma. All the blood samples were under storage of -80 °C at a central biorepository until laboratory analysis. Serum 25(OH)D concentrations (ng/ml), CTx (ng/ml) and total OC (ng/ml) were analyzed in a central laboratory using chemiluminescence assays on the Immunodiagnostic Systems iSYS (IDS-iSYS) automated analyser (Immunodiagnostic Systems GmbH, Frankfurt, Germany). IDS-iSYS 25-Hydroxy Vitamin D^{s}, CTX-I (CrossLaps®) and N-MID® Osteocalcin assay were used, respectively.

Vitamin D status was defined as sufficient or insufficient based on serum 25(OH)D concentrations at a cut-off of 20 ng/ml (= 50 nmol/l) as defined by the Institute of Medicine (IOM) (5). Serum CTx (ng/ml) was converted to the unit of pg/ml (1 ng/ml = 1000 pg/ml) in order to have better interpretation of the regression coefficients.

Bone stiffness index

Calcaneal QUS measurements were performed on both the right and left foot for each child using the well-established device Lunar Achilles Insight (GE Healthcare, Milwaukee, WI) (21). The calcaneus can represent bone metabolic changes since it consists of 90 % trabecular bone and has a high turnover rate. Besides, it is the only validated skeleton site for QUS devices to predict fracture risk according to the International Society for Clinical Densitometry (ISCD) (22). Other comparable Lunar Achilles devices have also been used in children and adolescents, and showed to be useful measures of bone development compared with DXA-derived bone mass (23, 24). In a sub-sample of 60 children aged 5.6-9.3 years from the IDEFCIS baseline survey, a reliability study was conducted suggesting the root-mean-square coefficient of variation (CV_{RMS}) for the reproducibility of repeated SI measurements within a QUS device on the left foot was 7.2%, and on the right foot was 9.2% (25). Before recording each measurement, the preview image of the

heel was displayed on the screen with the default region of interest (ROI) positioned correctly. Two different sizes of foot adapters were used to place the heels appropriately into the ROI. The Lunar Achilles OsteoReport Software was used to calculate SI from the parameters speed of sound (SOS, m/s) and broadband ultrasound attenuation (BUA, dB/MHz): SI = (0.67 * BUA) + (0.28 * SOS) - 420. Higher SI values indicate better bone health status. The mean SI value of the left and the right foot was calculated for each participant and further transformed to a sex-, age- and height-specific percentile based on the IDEFICS/I.Family reference population (25).

Co-variables

Information on PA, screen time duration and consumption frequency of dairy products were proxy-reported by parents for children up to 11 years, or self-reported by adolescents aged 12 years and older. PA was calculated by hours per week the participant spent doing sport in sports clubs. Screen time duration was calculated by hours per week from usually hours of watching TV/videos/DVDs and playing on a computer/game console for weekdays and weekend days. Consumption frequency of dairy products including milk, yoghurt and cheese during the last 4 weeks was collected using a validated and reproducibility tested food frequency questionnaire and expressed as frequency per week (26).

In a subgroup of participants, dietary intake of the previous 24 hours was assessed using a validated computer-based 24-h dietary recall. This computer software offered country-specific foods with photographs of standardized portion sizes and probing questions for usual foods and mixed meal components (27). Parents were asked to complete for their children below the age of 11 years, children aged 11 years and older completed independently. Participants were encouraged to repeatedly complete 24-h dietary recalls. Meals, drinks and snacks for children who had lunch at school were complemented by a standardized observer sheet completed by trained personnel. Simple foods or European homogeneous multi-ingredient food items were

linked to the German food composition table (German Nutrient Data Base, Version II.3.1); usual calcium intake (mg/day) was further estimated based on the U.S. National Cancer Institute Method (28). This method not only allows including the covariates age, sex, consumption frequency of dairy products and body mass index (BMI) to improve estimates, but also corrects for the variance inflation caused by the intra-individual daily variations even if repeated recalls are only available in a subgroup. According to the population reference intake for calcium from the European Food Safety Authority (EFSA), we defined children with daily calcium intake of at least 450 mg for 1 to 3 years old, 800 mg for 4 to 10 years old and 1150 mg for 11 to 17 years old as sufficient (29).

In a subgroup of participants, objectively measured PA and sedentary time were available using uniaxial accelerometers (GT1M or ActiTrainer, ActiGraph, Pensacola, FL, USA) or a triaxial accelerometer (GT3x+, ActiGraph, Pensacola, FL, USA) with vertical axis outputs. The sensor units of these models are identical. Participants were instructed to wear the accelerometer on the right hip by means of an elastic belt to ensure close contact with the body, and it was only removed during water-based activities and bedtime. All accelerometer data were re-integrated to a 60s epoch. Non-wear time was calculated and further removed according to Choi et al (30), at least six hours of wear time were considered as a valid day. Participants who had at least two valid weekdays and one valid weekend day of data were included in the analyses. More details of accelerometer data processing in the IDEFICS/I.Family cohort can be found elsewhere (31). Standard cut-points for counts per minute (cpm) were used to define the mean daily percentage of time spent at various intensities: sedentary time (≤100 cpm), light PA (101-2295 cpm), and moderate-to-vigorous PA (MVPA; ≥2296 cpm) for children and adolescents (32). Average of minutes per day was used for sedentary time and MVPA. According to the WHO guidelines on physical activity and sedentary behavior for children and adolescents, participants who had at least an average of 60 minutes per day were defined as having enough MVPA (33).

Height was measured to the nearest 0.1 cm using the standard clinical Seca 225 stadiometer (Seca, Hamburg, Germany); weight was measured to the nearest 0.1 kg using the BC420 SMA scale (Tanita, Amsterdam, The Netherlands). Height and weight were further transformed to age- and sex-specific z-scores based on Cole et al (34). BMI (kg/m^2) was calculated as body weight divided by squared body height, overweight/obesity was further defined based on the extended International Obesity Task Force (IOTF) BMI criteria (35).

Other confounders

Child sex, age, and parental educational level were reported by parents. The highest educational level of parents was used as an indicator for family socioeconomic status according to the International Standard Classification of Education (ISCED) and categorized into low (ISCED 0-2), medium (ISCED 3-5) and high (ISCED 6-8) (36). Not only vitamin D synthesis, but also immunomodulation, synthesis of hormones responsible for circadian rhythm, and mental health benefit from exposure to sunlight radiation, which may also result in bone-related changes (37, 38). Hence, we considered ultraviolet radiation index (UVI) of the month previous to the month of blood sampling as a proxy indicator for sunlight exposure (39).

Statistical Methods

All analyses were carried out using SAS software (V9.4; SAS Institute Inc, Cary, North Carolina, USA). Simple descriptive statistics (means, standard deviations (SDs) and frequencies) were presented for three main samples and three sub-samples; Pearson's correlations (r) between 25(OH)D, covariates and bone health indicators were calculated with 95% confidence intervals (CIs).

Linear mixed-effects models (SAS procedure PROC MIXED) were used to analyze the cross-sectional associations of serum 25(OH)D with CTx, OC and SI percentiles; a random effect for country was added to account for cluster effects; regression coefficients (β) and 95%CIs were

calculated. In the exploratory stages, all the analyses have been separately conducted by waves (wave 1 and 3) and age groups (preschool children: 2 to < 6 years, primary school children: 6 to < 12 years and adolescents: 12 to 15 years) in the first place, and then pooled in the final models if the effect sizes and directions of the associations were comparable among groups. A repeat statement in PROC MIXED was further added in the models when participants provided data in both wave 1 and wave 3. Sex, age, parental educational level, UVI and weight z-scores were taken into consideration as confounders, height z-scores were additionally adjusted for OC and CTx.

In the analysis of main samples, continuous 25(OH)D concentration (ng/ml), time spent in sports club (hours/week), screen time duration (hours/week) and consumption frequency of dairy products (frequency/week) were first included (Model 1). Further, vitamin D status (≥ 20 ng/ml vs. < 20 ng/ml) and weight status (thin/normal weight vs. overweight/obesity) were included instead with the confounder of weight z-scores removed (Model 2). The interaction term between weight status and vitamin D status was additionally tested and stratified based on model 2. In the analysis of sub-samples, continuous 25(OH)D concentration (ng/ml), accelerometer-measured MVPA (min/day), sedentary time (min/day) and usual calcium intake (mg/day) were first included (Model 3). Further, vitamin D status, MVPA level (≥ 60 min/day vs. < 60 min/day) and calcium intake level (sufficiency vs. insufficiency) were included instead (Model 4). The interaction terms between vitamin D status, MVPA level and calcium intake level were additionally investigated and stratified based on model 4.

Results

In total, there are three main samples and three sub-samples in the present study. The main samples consist of 1171 children for serum OC, 1752 children with 2481 observations for serum CTx, and 998 children with 1123 observations for SI, respectively. The sub-sample consist of

400 children for serum OC, 1013 children with 1165 observations for serum CTx, and 594 children with 645 observations for SI (Figure 1). The demographic characteristics of each sample are shown in Table 1.

Table 1 Demographic characteristics of main samples [1] and sub-samples [2] for each bone health indicators

	Serum OC		Serum CTx		SI percentiles	
	Main sample	Sub-sample	Main sample	Sub-sample	Main sample	Sub-sample
	N=1171	N=400	N=2481	N=1165	N=1123	N=645
Age (Mean, SD)	6.1(1.8)	6.1(1.9)	9.2(3.4)	9.8(3.3)	9.7(3.3)	9.0(3.4)
Sex (N, %)						
Boys	609(52.0)	214(53.5)	1246(50.2)	594(51.0)	327(50.7)	572(50.9)
Girls	562(48.0)	186(46.5)	1235(49.8)	571(49.0)	318(49.3)	551(49.1)
Parental educational level (N, %)						
Low	31(2.7)	10(2.5)	82(3.3)	36(3.10)	21(3.3)	44(3.9)
Medium	484(41.3)	150(37.5)	1064(42.9)	476(40.9)	287(44.5)	501(44.6)
High	656(56.0)	240(60.0)	1335(53.8)	653(56.0)	337(52.2)	578(51.5)
Country (N, %)						
Belgium	76(6.5)	10(2.5)	132(5.3)	69(5.9)	8(1.2)	42(3.7)
Cyprus	24(2.1)	4(1.0)	45(1.8)	5(0.4)	0	0
Estonia	210(17.9)	134(33.5)	414(16.7)	278(23.9)	158(24.5)	220(19.6)
Germany	148(12.6)	54(13.5)	376(15.2)	181(15.5)	146(22.6)	291(25.9)
Hungary	291(24.9)	55(13.8)	587(23.7)	145(12.5)	39(6.1)	148(13.2)
Italy	63(5.4)	8(2.0)	224(9.0)	132(11.3)	94(14.6)	145(12.9)
Spain	203(17.3)	80(20.0)	383(15.4)	230(19.7)	179(27.8)	212(18.9)
Sweden	156(13.3)	55(13.8)	320(12.9)	125(10.7)	21(3.3)	65(5.8)

Footnote: 1. main samples consist of children with full information on self-reported physical activity, sedentary behavior, consumption frequency of dairy products and each bone health outcome; 2. sub-samples consist of children had accelerometer data, calcium intake measured using 24-h dietary recall and each bone health outcome; OC osteocalcin, CTx C-terminal telopeptides of type I collagen, SI stiffness index

In the main samples, the mean values of OC, CTx and SI percentiles were 87.3 (SD = 32.1) ng/ml, 1976.9 (SD = 784.6) pg/ml and 53.9 (SD = 27.8), respectively. The proportion of individuals with vitamin D insufficiency and with overweight/obesity was 61.8% and 13.2% in OC sample, 63.0% and 18.2% in CTx sample, and 67.9% and 18.7% in the SI sample, respectively. In addition, OC and CTx were positively correlated (r = 0.54, 95%CI 0.49, 0.58, N = 1162), and both of them were inversely related to SI percentiles (r = -0.16, 95%CI -0.24, -0.08, N = 573, and r = -0.07, 95%CI -0.13, -0.01, N = 1120, respectively). In the sub-samples, the

mean values of serum OC, and CTx and SI percentiles were 86.4 (SD = 29.6) ng/ml, 2113.8 (SD = 834.7) pg/ml and 53.5 (SD = 27.7), respectively. The proportions of individuals with vitamin D < 20 ng/ml, with calcium intake insufficiency, and with MVPA < 60 min/day were 65.0%, 49.3% and 67.0% in OC sub-sample, 64.6%, 73.7% and 68.7% in CTx subsample, and 69.5%, 74.4% and 69.6% in SI sub-sample, respectively. Other description of exposure and covariates, as well as their correlations with outcomes are shown in Table 2.

Table 2 Descriptive characteristics of exposure and covariates, and their Pearson's correlations with each bone health indicators

	Serum OC		Serum CTx		SI percentiles	
	Mean (SD)	r (95%CI)	Mean (SD)	r (95%CI)	Mean (SD)	r (95%CI)
Main Sample [1]	N=1171		N=2481		N=1123	
Serum 25(OH)D (ng/ml)	18.0(6.1)	-0.07(-0.12,-0.01)	18.2(6.6)	-0.08(-0.12,-0.04)	17.1(6.1)	0.11(0.06,0.17)
Sports clubs (hours/week)	1.3(1.8)	0.12(0.07,0.18)	2.0(2.4)	0.19(0.15,0.23)	1.9(2.2)	0.02(-0.04,0.08)
Screen time (hours/week)	11.5(7.2)	0.12(0.06,0.17)	14.1(9.3)	0.11(0.08,0.15)	13.3(8.3)	-0.01(-0.07,0.04)
Dairy intake (frequency/week)	25.8(12.3)	0.02(-0.04,0.08)	25.4(15.3)	-0.04(-0.08,0.002)	25.1(14.9)	0.04(-0.03,0.09)
Height z-scores	0.6(1.0)	0.20(0.14,0.25)	0.60(1.0)	0.14(0.10,0.18)	0.6(1.0)	-0.03(-0.09,0.02)
Weight z-scores	0.3(1.0)	0.17(0.12,0.23)	0.50(1.1)	0.04(0.003,0.08)	0.5(1.0)	0.04(-0.01,0.10)
Sub-sample [2]	N=400		N=1165		N=645	
Serum 25(OH)D (ng/ml)	17.5(6.3)	-0.10(-0.20,-0.002)	17.9(6.8)	-0.07(-0.13-0.01)	17.0(6.0)	0.10(0.02,0.17)
MVPA (min/day)	52.6(20.8)	0.08(-0.01,0.18)	51.8(21.1)	0.03(-0.03,0.08)	50.6(20.5)	0.07(-0.01,0.15)
Sedentary time (min/day)	408.2(144.8)	0.003(-0.09,0.10)	518.5(139.7)	0.14(0.08,0.20)	519.5(143.0)	0.13(0.06,0.21)
Usual calcium intake (mg/day)	777.8(191.8)	-0.06(-0.15,0.04)	730.1(207.0)	-0.02(-0.08,0.03)	715.6(190.9)	0.07(-0.004,0.15)
Height z-scores	0.6(1.1)	0.20(0.11,0.30)	0.6(1.0)	0.17(0.11,0.22)	0.6(1.0)	0.02(-0.05,0.10)
Weight z-scores	0.3(1.0)	0.17(0.08,0.27)	0.5(1.1)	0.05(-0.004,0.11)	0.5(1.0)	0.14(0.06,0.21)

Footnote: 1. main samples consist of children with full information on self-reported physical activity, sedentary behavior, consumption frequency of dairy products and each bone health outcome; 2. sub-samples consist of children had accelerometer data, calcium intake measured using 24-h dietary recall and each bone health outcome; OC osteocalcin, CTx C-terminal telopeptides of type I collagen, SI stiffness index, 25(OH)D 25-hydroxyvitamin D, MVPA Moderate-to-vigorous physical activity

The associations of serum 25(OH)D with CTx and OC were stratified by age groups (Table 3). In preschool children, we observed an inverse association between serum 25(OH)D and CTx (β = -8.52, 95%CI -15.36, -1.68) (Model 1). Moreover, participants with MVPA ≥ 60 min/day had higher OC compared to those with MVPA < 60 min/day (β = 8.67, 95%CI 0.75, 16.58) (Model 4). In primary school children, we observed higher CTx in participants with thin/normal weight compared to those with overweight/obesity (β = 180.36, 95%CI 102.93, 257.78) (Model 2). Moreover, weight status had an interactive effect on the association between vitamin D status and CTx, suggesting an inverse association in participants with thin/normal weight (β = -47.95, 95%CI -120.87, 24.98) but a positive association in participants with overweight/obesity (β =

107.94, 95%CI -41.37, 257.26). In adolescents, time spent in sports clubs (β = 25.50, 95%CI 2.58, 48.42) was positively associated with CTx, while screen time duration (β = -7.73, 95%CI -13.66, -1.81) was inversely associated (Model 1). Moreover, higher CTx was also observed in participants with thin/normal weight compared to whom with overweight/obesity (β = 290.71, 95%CI 133.24, 448.18) (Model 2). Overall, consumption frequency of dairy intake (β = -1.64, 95%CI -3.28, -0.002, Model 1) and serum 25(OH)D (β = -7.09, 95%CI -13.42, -0.75, Model 3) were inversely associated with CTx; children who met the population reference intake for calcium had lower CTx compared to whom did not (β = -116.70, 95%CI -204.39, -29.01, Model 4).

The association between 25(OH)D and SI percentiles was stratified by waves (Table 4). In wave 1, children with serum 25(OH)D ≥ 20 ng/ml had lower SI percentiles compared to those with 25(OH)D < 20 ng/ml (β = -5.20, 95%CI -9.77, -0.62, Model 2). A positive association was observed between sedentary time and SI percentiles (β = 0.04, 95%CI 0.01, 0.07, Model 3). In wave 3, time spent in sports clubs (β = 1.28, 95%CI 0.37, 2.18) and consumption frequency of dairy intake (β = 0.15, 95%CI 0.02, 0.28) were positively associated with SI percentiles (Model 1). Moreover, children with thin/normal weight had lower SI percentiles compared to those with overweight/obesity (β = -13.64, 95%CI -18.92, -8.36) (Model 2). In addition, MVPA (β = 0.20, 95%CI 0.06, 0.34) and usual calcium intake (β = 0.02, 95%CI 0.003, 0.03) were positively associated with SI percentiles (Model 3), and children with MVPA ≥ 60 min/day had higher SI percentiles compared to those with MVPA < 60 min/day (β = 6.24, 95%CI 0.21, 12.28). Overall, we observed an interaction of MVPA with the association between vitamin D status and SI percentiles, suggesting a positive association in children with MVPA ≥ 60 min/day (β = 12.14, 95%CI 4.28, 20.00) while a negative association in children with MVPA < 60 min/day (β = -6.05, 95%CI 11.85, -0.26).

Table 3 Associations of serum 25(OH)D and covariates with bone formation marker OC and bone resorption marker CTx, stratified by age groups

	Serum OC			Serum CTx			
	2 to < 6 years	6 to <12 years	Total [1]	2 to < 6 years	6 to <12 years [1]	12 to 15 years	Total [1]
	β (95%CI)	β (95%CI)	β (95%CI)	β (95%CI)	β (95%CI)	β (95%CI)	β (95%CI)
Main sample [2]	N=510	N=661	N=1171	N=512	N=1293	N=676	N=2481
Model 1							
Serum 25(OH)D (ng/ml)	0.07(-0.38,0.52)	-0.17(-0.60,0.27)	0.01(-0.30,0.33)	**-8.52(-15.36,-1.68)**	0.76(-4.60,6.11)	-7.53(-17.70,2.64)	-4.00(-8.16,0.17)
Sports clubs (hours/week)	1.45(-0.75,3.65)	0.14(-1.05,1.33)	0.21(-0.82,1.24)	13.38(-19.97,46.73)	-6.89(-21.56,7.78)	**25.50(2.58,48.42)**	**15.75(3.73,27.76)**
Screen time (hours/week)	0.09(-0.28,0.46)	0.18(-0.15,0.51)	0.12(-0.12,0.37)	0.32(-5.29,5.93)	1.66(-2.49,5.81)	**-7.73(-13.66,-1.81)**	1.08(-1.98,4.14)
Dairy intake (frequency/week)	-0.02(-0.22,0.19)	-0.04(-0.22,0.14)	-0.02(-0.15,0.12)	-1.57(-4.49,1.34)	-1.22(-3.44,1.01)	-2.11(-5.36,1.13)	**-1.64(-3.28,-0.002)**
Model 2 [3]							
25(OH)D ≥ 20 ng/ml vs. < 20 ng/ml (ref)	0.94(-4.40,6.28)	-2.86(-7.85,2.14)	-0.34(-4.02,3.33)	-67.34(-148.83,14.15)	-9.82(-75.50,55.86)	-28.41(-175.26,118.44)	-14.95(-65.27,35.37)
Thin/normal weight vs. overweight/obesity (ref)	-6.56(-16.12,2.99)	4.47(-1.45,10.38)	0.63(-4.35,5.62)	-13.15(-158.53,132.24)	**180.36(102.93,257.78)**	**290.71(133.24,448.18)**	**147.21(81.80,212.62)**
Sub-sample [4]	N=182	N=218	N=400	N=184	N=604	N=377	N=1165
Model 3							
Serum 25(OH)D (ng/ml)	0.25(-0.32,0.82)	-0.38(-1.08,0.33)	-0.13(-0.60,0.33)	-4.17(-14.61,6.27)	-5.90(-13.65,1.85)	-13.86(-27.87,0.14)	**-7.09(-13.42,-0.75)**
MVPA (min/day)	0.11(-0.10,0.32)	-0.02(-0.21,0.17)	0.04(-0.10,0.18)	1.72(-2.10,5.54)	2.23(-0.03,4.48)	-2.19(-7.07,2.70)	1.43(-0.43,3.30)
Sedentary time (min/day)	-0.02(-0.05,0.01)	-0.01(-0.04,0.02)	-0.02(-0.04,0.001)	-0.15(-0.63,0.33)	-0.32(-0.71,0.07)	-0.72(-1.72,0.28)	-0.31(-0.64,0.02)
Usual calcium intake (mg/day)	-0.02(-0.03,0.004)	-0.01(-0.03,0.01)	-0.01(-0.03,0.005)	-0.30(-0.64,0.05)	-0.21(-0.45,0.03)	-0.01(-0.44,0.41)	-0.19(-0.39,0.01)
Model 4							
25(OH)D ≥ 20 ng/ml vs. < 20 ng/ml (ref)	3.53(-3.77,10.83)	-5.88(-14.54,2.78)	-1.68(-7.48,4.12)	-25.40(-160.04,109.23)	-50.89(-151.60,49.81)	-128.64(-321.85,64.58)	-40.08(-121.15,40.99)
MVPA ≥ 60 min/day vs. < 60 min/day (ref)	**8.67(0.75,16.58)**	-3.26(-11.63,5.11)	1.94(-3.80,7.68)	85.05(-60.66,230.77)	36.04(-61.65,133.73)	-92.38(-311.45,126.69)	33.60(-46.43,113.64)
Calcium intake sufficiency vs. insufficiency (ref) [5]	-2.96(-10.85,4.94)	-1.78(-10.10,6.54)	-3.99(-9.72,1.74)	-133.01(-278.91,12.90)	-56.39(-158.30,45.52)	-66.21(-555.29,422.86)	**-116.70(-204.39,-29.01)**

Footnote: Linear mixed-effects models were used with sex, age, parental educational level, ultraviolet radiation index, height and weight z-scores included as confounders, a random effect for countries was taken into account in all models; 1. a repeat statement was added in the model to account for repeated measurements; 2. main samples consist of children with full information on self-reported physical activity, sedentary behavior, consumption frequency of dairy products and each bone turnover marker; 3. weight z-score was removed in the model, weight status was defined according to cole at el.; 4. sub-samples consist of children had accelerometer data, calcium intake measured using 24-h dietary recall and each bone turnover marker; 5. daily calcium intake was classified into sufficiency and insufficiency at cut-offs of 450 mg for 1 to 3 years old, 800 mg for 4 to 10 years old and 1150 mg for 11 to 17 years old according to the population reference intake for calcium from European Food Safety Authority; OC osteocalcin, CTx C-terminal telopeptides of type I collagen, 25(OH)D 25-hydroxyvitamin D, MVPA Moderate-to-vigorous physical activity

Table 4 Associations of serum 25(OH)D and covariates with bone stiffness index percentiles, stratified by examination waves

	Wave 1	Wave 3	Total [1]
	β (95%CI)	β (95%CI)	β (95%CI)
Main sample [2]	N=577	N=546	N=1123
Model 1			
Serum 25(OH)D (ng/ml)	-0.26(-0.65,0.13)	0.38(-0.05,0.81)	-0.10(-0.40,0.20)
Sports clubs (hours/week)	0.06(-1.16,1.29)	**1.28(0.37,2.18)**	0.11(-0.64,0.86)
Screen time (hours/week)	0.01(-0.33,0.34)	-0.10(-0.35,0.15)	-0.06(-0.27,0.15)
Dairy intake (frequency/week)	-0.04(-0.20,0.11)	**0.15(0.02,0.28)**	0.06(-0.04,0.17)
Model 2 [3]			
25(OH)D ≥ 20 ng/ml v.s. < 20 ng/ml (ref)	**-5.20(-9.77,-0.62)**	4.15(-1.11,9.42)	-2.30(-5.83,1.22)
Thin/normal weight v.s. overweight/obesity (ref)	5.47(-0.59,11.53)	**-13.64(-18.92,-8.36)**	-3.31(-7.51,0.89)
Sub-sample [4]	N=243	N=402	N=645
Model 3			
Serum 25(OH)D (ng/ml)	-0.27(-0.83,0.29)	0.18(-0.32,0.67)	-0.09(-0.47,0.30)
MVPA (min/day)	0.16(-0.01,0.34)	**0.20(0.06,0.34)**	0.06(-0.05,0.17)
Sedentary time (min/day)	**0.04(0.01,0.07)**	-0.02(-0.04,0.01)	0.01(-0.01,0.03)
Usual calcium intake (mg/day)	-0.003(-0.02,0.02)	**0.02(0.003,0.03)**	0.01(-0.003,0.02)
Model 4			
25(OH)D ≥ 20 ng/ml v.s. < 20 ng/ml (ref)	-4.68(-11.94,2.58)	3.59(-2.33,9.51)	-0.55(-5.27,4.17)
MVPA ≥ 60 min/day v.s. < 60 min/day (ref)	4.93(-1.88,11.73)	**6.24(0.21,12.28)**	2.43(-2.16,7.02)
Calcium intake sufficiency v.s. insufficiency [5]	2.03(-5.02,9.09)	6.39(-2.30,15.08)	4.28(-1.31,9.88)

Footnote: Linear mixed-effects models were used with sex, age, parental educational level, ultraviolet radiation index and weight z-scores included as confounders, a random effect for countries was taken into account in all models; 1. a repeat statement was added in the model to account for repeated measurements; 2. main samples consist of children with full information on self-reported physical activity, sedentary behavior, consumption frequency of dairy products and bone stiffness index; 3. weight z-score was removed in the model, weight status was defined according to cole at el.; 4. sub-samples consist of children had accelerometer data, calcium intake measured using 24-h dietary recall and bone stiffness index; 5. daily calcium intake was classified into sufficiency and insufficiency at cut-offs of 450 mg for 1 to 3 years old, 800 mg for 4 to 10 years old and 1150 mg for 11 to 17 years old according to the population reference intake for calcium from European Food Safety Authority; 25(OH)D 25-hydroxyvitamin D, MVPA Moderate-to-vigorous physical activity

Discussion

In the present study, we found that serum 25(OH)D and consumption frequency of dairy products were inversely associated with the bone resorption marker CTx. Moreover, children who met the population reference intake for calcium had lower CTx compared to who did not. Furthermore, MVPA was a moderator in the association between 25(OH)D and SI, suggesting that 25(OH)D sufficiency was a protective factor for calcaneal SI only in children meeting the MVPA recommendation for average 60 min/day. The stratified results suggested that meeting

the MVPA recommendation was associated with higher OC in pre-school children. In primary school children, the interaction of weight status on the association between vitamin D status and CTx suggested an inverse association in thin/normal weight group but a positive association in overweight/obese group. In adolescents, we found a positive association of time spent in sports clubs and an inverse association of screen time duration with CTx. In primary school children and adolescents, participants with thin/normal weight had higher CTx but lower SI percentiles than those with overweight/obesity. Moreover, time spent in sports clubs and MVPA, as well as consumption frequency of dairy intake and usual calcium intake showed consistently positive associations with SI percentiles.

Our results suggested a stronger inverse association of serum 25(OH)D with bone resorption marker CTx in contrast to bone formation marker OC. This finding is in line with previous observational studies which were conducted in Finnish peri-pubertal girls and German pre-pubertal children (40, 41). Our observation is also in accordance to an intervention study conducted in children with vitamin D deficiency, which suggested that vitamin D supplementation resulted in a stronger decrease of bone resorption compared to bone formation (42). Considering the inverse correlations between bone turnover markers and calcaneal SI, our finding demonstrates that increased 25(OH)D along with reduced bone turnover especially for bone resorption may be beneficial for improving bone stiffness.

There is a general agreement that the threshold for vitamin D sufficiency should be based on the serum 25(OH)D concentration required to suppress parathyroid hormone (PTH) secretion (5). A cross-sectional study conducted in Northern Irish adolescents found the PTH plateau occurred at a 25(OH)D concentration of approximately 60 nmol/l in girls, while no PTH plateau was observed in boys (43). However, another study conducted in Chinese adolescents reported that the thresholds of 25(OH)D levels having beneficial effects on PTH, BMD and other bone remodeling markers were 20-37 nmol/l in girls and 33-39 nmol/l in boys (44). Up to now, there

is still lack of consensus on serum 25(OH)D concentration thresholds to define deficient and sufficient vitamin D status (5).

In the present study, a cut-off of 20 ng/ml (= 50 nmol/l) for serum 25(OH)D concentration was used to indicate sufficiency, which has been commonly used in pediatric populations (45). We found children with vitamin D sufficiency tended to have lower CTx compared to those with insufficiency, albeit the differences were small. In the sensitivity analysis, we used the cut-off of 30 ng/ml (=75 nmol/l) and observed a larger but still not statistically significant differences. On the contrary, we found that vitamin D status tended to positively associate with CTx in primary school children with overweight/obesity. The possible explanation for this contradicting association could be the coexistence of low vitamin D status and low CTx in children with overweight/obesity. It has been suggested that obese children had lower bone turnover and 25(OH)D as well as higher PTH level (46). Our findings support that it is important considering the modulatory effect of weight status and age on bone turnover makers in further studies.

We found that vitamin D sufficiency was a protective factor for calcaneal SI only in children meeting the MVPA guideline for average 60 min/day. This result is comparable with the finding from the HELENA study, albeit this multi-country study used a cut-off of 75 nmol/l (47). Even though in the IDEFICS case-control study, odds ratios for poor SI were not statistically significant across the tertiles of 25(OH)D, the highest risk for poor SI was observed for low MVPA (< 5.4% of total wearing time) combined with low 25OHD (< 43.0 nmol/l) using the median as cut-off values (13). Another cross-sectional study conducted in 0 to 6 year-old children reported a high risk for low QUS-derived SOS at mid-tibia in low 25(OH)D (< 20 ng/ml) (48). Nevertheless, we found children with sufficient vitamin D had lower SI compared to those with insufficient vitamin D, and sedentary time was positively associated with SI in the first examination. The possible explanation for these unexpected directions could be the observed interplays of MVPA and weight status among these associations. In addition, a longitudinal

study followed pre-pubertal girls for a period of up to 9 years also reported an inverse association between 25(OH)D and BMC (7). In the future, more longitudinal and experimental studies are needed to investigate the casual inference and pathways among these associations.

The osteogenic effect of PA has been demonstrated in previous IDEFICS/I.Family studies (49, 50). In the present study, we also observed consistently positive associations of time spent in sports clubs and MVPA with SI. Moreover, in preschool children, we found having at least 60 min/day of MVPA was associated with increased OC. In adolescents, time spent in sports clubs was positively associated CTx while screen time duration was negatively associated with CTx. Evidence suggests that children at age 7 years had obtained 70% of maximal observed height, and the bone mineral accretion was highest during early puberty (51). Therefore, the increase of bone turnover makers related to bone modeling may be beneficial for bone growth in these critical periods of life. Our results support the important windows of opportunities during pre-school period and pubertal period for behavioral interventions on bone health.

We found that dairy products consumption and calcium intake were independently associated with reduced bone resorption and with increased SI percentiles. Dietary requirements for calcium during childhood are mainly determined by skeletal growth, and the milk and other dairy foods are the primary source of calcium in the western diet tradition. Numerous randomized controlled trials in children using calcium supplementation, calcium-fortified foods or dairy foods reported positive effects on bone mass at several skeletal sites (52). It is also suggested that reduced bone turnover in bone remodeling instead of modeling accounts for the benefits of calcium supplementation on BMD in children (53). Two milk intervention studies also reported the short- and long-term reductions in bone turnovers, and suggested that these effects may be mediated by reduced PTH secretion (54, 55). Although there is strong and abundant evidence indicating the important role of calcium intake, the majority of participants did not meet the population reference intake for calcium in the present study.

The main strength of the present study is the large-scale population-based sample of children aged 2 to 15 years from 8 European countries. Moreover, serum bone turnover markers as well as QUS-derived SI were measured as indicators of bone health, which are rarely examined in healthy pediatric populations. In addition, a wide range of potential influential factors using both subjective and objective measures were considered. However, there are some limitations to acknowledge. First, the cross-sectional design did not allow us to make causal inference. Second, the availability of variables of interest reduced the sample size and may result in lacking statistical power. Third, the information of pubertal stages is unavailable. Instead, we used the menarche for girls and voice change for boys as a proxy indicator in the third examination to define pre-pubertal and pubertal, and computed missing values into pre-pubertal for the first examination since most of the children (98.7%) was younger than 9 years. However, no modifying effects were observed in the exploratory stages.

Conclusions

In conclusion, we observed that serum 25(OH)D, consumption frequency of dairy intake, and usual calcium intake were inversely associated with serum CTx. In addition, meeting the MVPA recommendation was associated with increased OC in pre-school children, while time spent in sports club was positively but screen time duration and weight status were negatively associated with CTx in adolescents, which emphasize the subclinical impacts of these modifiable factors on bone modelling particularly in these two critical periods of life. Moreover, our study supports the important roles of physical activity, dairy products and calcium intake on bone stiffness. In addition, weight status and average daily MVPA have interactive effects on the associations of vitamin D status with bone resorption and bone stiffness, respectively. Therefore, future intervention studies on vitamin D supplementations and bone health should combine with improving MVPA level and take weight status and age into consideration.

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Conflicts of interests

Lan Cheng, Hermann Pohlabeln, Maike Wolters, Wolfgang Ahrens, Alfonso Siani, Toomas Veidebaum, Michael Tornaritis, Dénes Molnár, Gabriele Eiben, Monica Hunsberger, Stefaan De Henauw, Luis A Moreno, Antje Hebestreit have no potential conflicts of interest.

Authors' contributions

Lan Cheng, Wolfgang Ahrens and Antje Hebestreit designed the study including research conception and development of overall research plan; Hermann Pohlabeln and Maike Wolters supported the data analysis and interpretation; Alfonso Siani, Toomas Veidebaum, Michael Tornaritis, Dénes Molnár, Gabriele Eiben, Monica Hunsberger, Stefaan De Henauw, Luis A Moreno contributed to coordination and data collection; Lan Cheng performed statistical analysis and wrote the draft of manuscript; all the authors improved and approved the final manuscript; Lan Cheng and Antje Hebestreit had primary responsibility for the final content.

References

1. Cashman KD, Dowling KG, Skrabakova Z, Gonzalez-Gross M, Valtuena J, De Henauw S, et al. Vitamin D deficiency in Europe: pandemic? Am J Clin Nutr. 2016;103(4):1033-44.

2. Martineau AR, Jolliffe DA, Hooper RL, Greenberg L, Aloia JF, Bergman P, et al. Vitamin D supplementation to prevent acute respiratory tract infections: systematic review and meta-analysis of individual participant data. BMJ. 2017;356:i6583.

3. Mirhosseini N, Rainsbury J, Kimball SM. Vitamin D Supplementation, Serum 25(OH)D Concentrations and Cardiovascular Disease Risk Factors: A Systematic Review and Meta-Analysis. Front Cardiovasc Med. 2018;5:87.

4. Jamilian H, Amirani E, Milajerdi A, Kolahdooz F, Mirzaei H, Zaroudi M, et al. The effects of vitamin D supplementation on mental health, and biomarkers of inflammation and oxidative stress in patients with psychiatric disorders: A systematic review and meta-analysis of randomized controlled trials. Prog Neuropsychopharmacol Biol Psychiatry. 2019;94:109651.

5. Moon RJ, Davies JH, Cooper C, Harvey NC. Vitamin D, and Maternal and Child Health. Calcif Tissue Int. 2020;106(1):30-46.

6. Pekkinen M, Viljakainen H, Saarnio E, Lamberg-Allardt C, Makitie O. Vitamin D is a major determinant of bone mineral density at school age. PloS one. 2012;7(7):e40090.

7. Breen ME, Laing EM, Hall DB, Hausman DB, Taylor RG, Isales CM, et al. 25-hydroxyvitamin D, insulin-like growth factor-I, and bone mineral accrual during growth. J Clin Endocrinol Metab. 2011;96(1):E89-98.

8. Li J, Ding W, Cao J, Sun L, Liu S, Zhang J, et al. Serum 25-hydroxyvitamin D and bone mineral density among children and adolescents in a Northwest Chinese city. Bone. 2018;116:28-34.

9. Cashman KD, Hill TR, Cotter AA, Boreham CA, Dubitzky W, Murray L, et al. Low vitamin D status adversely affects bone health parameters in adolescents. Am J Clin Nutr. 2008;87(4):1039-44.

10. Hazell TJ, Pham TT, Jean-Philippe S, Finch SL, El Hayek J, Vanstone CA, et al. Vitamin D status is associated with bone mineral density and bone mineral content in preschool-aged children. J Clin Densitom. 2015;18(1):60-7.

11. Winzenberg T, Powell S, Shaw KA, Jones G. Effects of vitamin D supplementation on bone density in healthy children: systematic review and meta-analysis. Bmj. 2011;342:c7254.

12. Baroncelli GI. Quantitative ultrasound methods to assess bone mineral status in children: technical characteristics, performance, and clinical application. Pediatr Res. 2008;63(3):220-8.

13. Herrmann D, Pohlabeln H, Gianfagna F, Konstabel K, Lissner L, Mårild S, et al. Association between bone stiffness and nutritional biomarkers combined with weight-bearing exercise, physical activity, and sedentary time in preadolescent children. A case-control study. Bone. 2015;78:142-9.

14. Greenblatt MB, Tsai JN, Wein MN. Bone Turnover Markers in the Diagnosis and Monitoring of Metabolic Bone Disease. Clinical chemistry. 2017;63(2):464-74.

15. Kuchuk NO, van Schoor NM, Pluijm SM, Chines A, Lips P. Vitamin D status, parathyroid function, bone turnover, and BMD in postmenopausal women with osteoporosis: global perspective. Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research. 2009;24(4):693-701.

16. Dalskov S, Ritz C, Larnkjær A, Damsgaard CT, Petersen RA, Sørensen LB, et al. Associations between adiposity, hormones, and gains in height, whole-body height-adjusted bone size, and size-adjusted bone mineral content in 8- to 11-year-old children. Osteoporos Int. 2016;27(4):1619-29.

17. Kouda K, Ohara K, Nakamura H, Fujita Y, Iki M. Predicting bone mineral acquisition during puberty: data from a 3-year follow-up study in Hamamatsu, Japan. Journal of bone and mineral metabolism. 2017;35(2):185-91.

18. Lucas R, Martins A, Monjardino T, Caetano-Lopes J, Fonseca JE. Bone Markers Throughout Sexual Development: Epidemiological Significance and Population-Based Findings. In: Preedy VR, editor. Biomarkers in Bone Disease. Dordrecht: Springer Netherlands; 2016. p. 1-34.

19. Ahrens W, Bammann K, Siani A, Buchecker K, De Henauw S, Iacoviello L, et al. The IDEFICS cohort: design, characteristics and participation in the baseline survey. Int J Obes (Lond). 2011;35 Suppl 1:S3-15.

20. Ahrens W, Siani A, Adan R, De Henauw S, Eiben G, Gwozdz W, et al. Cohort Profile: The transition from childhood to adolescence in European children-how I.Family extends the IDEFICS cohort. Int J Epidemiol. 2017;46(5):1394-5j.

21. Economos CD, Sacheck JM, Wacker W, Shea K, Naumova EN. Precision of Lunar Achilles+ bone quality measurements: time dependency and multiple machine use in field studies. The British journal of radiology. 2007;80(959):919-25.

22. Krieg MA, Barkmann R, Gonnelli S, Stewart A, Bauer DC, Del Rio Barquero L, et al. Quantitative ultrasound in the management of osteoporosis: the 2007 ISCD Official Positions. J Clin Densitom. 2008;11(1):163-87.

23. Alwis G, Rosengren B, Nilsson JA, Stenevi-Lundgren S, Sundberg M, Sernbo I, et al. Normative calcaneal quantitative ultrasound data as an estimation of skeletal development in Swedish children and adolescents. Calcif Tissue Int. 2010;87(6):493-506.

24. Xu Y, Guo B, Gong J, Xu H, Bai Z. The correlation between calcaneus stiffness index calculated by QUS and total body BMD assessed by DXA in Chinese children and adolescents. Journal of bone and mineral metabolism. 2014;32(2):159-66.

25. Herrmann D, Intemann T, Lauria F, Mårild S, Molnár D, Moreno LA, et al. Reference values of bone stiffness index and C-terminal telopeptide in healthy European children. Int J Obes (Lond). 2014;38 Suppl 2:S76-85.

26. Lanfer A, Hebestreit A, Ahrens W, Krogh V, Sieri S, Lissner L, et al. Reproducibility of food consumption frequencies derived from the Children's Eating Habits Questionnaire used in the IDEFICS study. Int J Obes (Lond). 2011;35 Suppl 1:S61-8.

27. Hebestreit A, Wolters M, Jilani H, Eiben G, Pala V. Web-Based 24-h Dietary Recall: The SACANA Program. Instruments for health surveys in children and adolescents: Springer; 2019. p. 77-102.

28. Tooze JA, Kipnis V, Buckman DW, Carroll RJ, Freedman LS, Guenther PM, et al. A mixed-effects model approach for estimating the distribution of usual intake of nutrients: the NCI method. Stat Med. 2010;29(27):2857-68.

29. EFSA Panel on Dietetic Products N, Allergies. Scientific opinion on dietary reference values for calcium. EFSA Journal. 2015;13(5):4101.

30. Choi L, Liu Z, Matthews CE, Buchowski MS. Validation of accelerometer wear and nonwear time classification algorithm. Med Sci Sports Exerc. 2011;43(2):357-64.

31. Buck C, Eiben G, Lauria F, Konstabel K, Page A, Ahrens W, et al. Urban Moveability and physical activity in children: longitudinal results from the IDEFICS and I.Family cohort. Int J Behav Nutr Phys Act. 2019;16(1):128.

32. Evenson KR, Catellier DJ, Gill K, Ondrak KS, McMurray RG. Calibration of two objective measures of physical activity for children. Journal of sports sciences. 2008;26(14):1557-65.

33. Chaput JP, Willumsen J, Bull F, Chou R, Ekelund U, Firth J, et al. 2020 WHO guidelines on physical activity and sedentary behaviour for children and adolescents aged 5-17 years: summary of the evidence. Int J Behav Nutr Phys Act. 2020;17(1):141.

34. Cole TJ, Freeman JV, Preece MA. British 1990 growth reference centiles for weight, height, body mass index and head circumference fitted by maximum penalized likelihood. Stat Med. 1998;17(4):407-29.

35. Cole TJ, Lobstein T. Extended international (IOTF) body mass index cut-offs for thinness, overweight and obesity. Pediatr Obes. 2012;7(4):284-94.

36. Statistics UIf. International standard classification of education: ISCED 2011: UNESCO Institute for Statistics Montreal; 2012.

37. Powers JM, Murphy JEJ. Sunlight radiation as a villain and hero: 60 years of illuminating research. International journal of radiation biology. 2019;95(7):1043-9.

38. Tse BCY, Byrne SN. Lipids in ultraviolet radiation-induced immune modulation. Photochemical & photobiological sciences : Official journal of the European Photochemistry Association and the European Society for Photobiology. 2020;19(7):870-8.

39. WHO. UV radiation [Available from: https://www.who.int/uv/intersunprogramme/activities/uv_index/en/index3.html.

40. Lehtonen-Veromaa MK, Möttönen TT, Nuotio IO, Irjala KM, Leino AE, Viikari JS. Vitamin D and attainment of peak bone mass among peripubertal Finnish girls: a 3-y prospective study. Am J Clin Nutr. 2002;76(6):1446-53.

41. Thiering E, Brüske I, Kratzsch J, Hofbauer LC, Berdel D, von Berg A, et al. Associations between serum 25-hydroxyvitamin D and bone turnover markers in a population based sample of German children. Sci Rep. 2015;5:18138.

42. Marwaha RK, Garg MK, Mithal A, Gupta S, Shukla M, Chadha A. Effect of Vitamin D Supplementation on Bone Turnover Markers in Children and Adolescents from North India. Indian J Endocrinol Metab. 2019;23(1):27-34.

43. Hill TR, Cotter AA, Mitchell S, Boreham CA, Dubitzky W, Murray L, et al. Vitamin D status and parathyroid hormone relationship in adolescents and its association with bone

health parameters: analysis of the Northern Ireland Young Heart's Project. Osteoporos Int. 2010;21(4):695-700.

44. Wu F, Laslett LL, Zhang Q. Threshold Effects of Vitamin D Status on Bone Health in Chinese Adolescents With Low Calcium Intake. J Clin Endocrinol Metab. 2015;100(12):4481-9.

45. Misra M, Pacaud D, Petryk A, Collett-Solberg PF, Kappy M. Vitamin D deficiency in children and its management: review of current knowledge and recommendations. Pediatrics. 2008;122(2):398-417.

46. Geserick M, Vogel M, Eckelt F, Schlingmann M, Hiemisch A, Baber R, et al. Children and adolescents with obesity have reduced serum bone turnover markers and 25-hydroxyvitamin D but increased parathyroid hormone concentrations - Results derived from new pediatric reference ranges. Bone. 2020;132:115124.

47. Valtueña J, Gracia-Marco L, Vicente-Rodríguez G, González-Gross M, Huybrechts I, Rey-López JP, et al. Vitamin D status and physical activity interact to improve bone mass in adolescents. The HELENA Study. Osteoporos Int. 2012;23(8):2227-37.

48. Yu X, Zhang J, Yan C, Shen X. Relationships between serum 25-hydroxyvitamin D and quantitative ultrasound bone mineral density in 0-6 year old children. Bone. 2013;53(1):306-10.

49. Cheng L, Pohlabeln H, Ahrens W, Lauria F, Veidebaum T, Chadjigeorgiou C, et al. Cross-sectional and longitudinal associations between physical activity, sedentary behaviour and bone stiffness index across weight status in European children and adolescents. Int J Behav Nutr Phys Act. 2020;17(1):54.

50. Herrmann D, Buck C, Sioen I, Kouride Y, Marild S, Molnar D, et al. Impact of physical activity, sedentary behaviour and muscle strength on bone stiffness in 2-10-year-old children-cross-sectional results from the IDEFICS study. Int J Behav Nutr Phys Act. 2015;12:112.

51. McCormack SE, Cousminer DL, Chesi A, Mitchell JA, Roy SM, Kalkwarf HJ, et al. Association Between Linear Growth and Bone Accrual in a Diverse Cohort of Children and Adolescents. JAMA Pediatr. 2017;171(9):e171769.

52. Weaver CM, Gordon CM, Janz KF, Kalkwarf HJ, Lappe JM, Lewis R, et al. The National Osteoporosis Foundation's position statement on peak bone mass development and lifestyle factors: a systematic review and implementation recommendations. Osteoporos Int. 2016;27(4):1281-386.

53. Slemenda CW, Peacock M, Hui S, Zhou L, Johnston CC. Reduced rates of skeletal remodeling are associated with increased bone mineral density during the development of peak skeletal mass. Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research. 1997;12(4):676-82.

54. Budek AZ, Hoppe C, Michaelsen KF, Mølgaard C. High intake of milk, but not meat, decreases bone turnover in prepubertal boys after 7 days. Eur J Clin Nutr. 2007;61(8):957-62.

55. Zhu K, Du X, Cowell CT, Greenfield H, Blades B, Dobbins TA, et al. Effects of school milk intervention on cortical bone accretion and indicators relevant to bone metabolism in Chinese girls aged 10-12 y in Beijing. Am J Clin Nutr. 2005;81(5):1168-75.