Bone Stiffness and its Association with Physical Activity Behaviour in Children

An Epidemiological Perspective

Dissertation

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Abbreviations

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BMC	Bone mineral content
aBMD	Areal bone mineral density
BUA	Broadband ultrasound attenuation
CTX	Carboxy-terminal telopeptide of type I collagen (β-crosslaps)
CV	Coefficient of variation
CV_{RMS}	Root-mean-squared coefficient of variation
DXA	Dual-energy x-ray densitometry
FDA	The Food and Drug Administration
IDEFICS	**I**dentification and prevention of **D**ietary- and lifestyle- induced health **EF**fects in **C**hildren and infant**S**
IFCC	International Federation of Clinical Chemistry and Laboratory Medicine
IOF	International Osteoporosis Foundation
ISCD	International Society of Clinical Densitometry
MVPA	Moderate-to-vigorous physical activity
p.	Page
PA	Physical activity
pQCT	Peripheral quantitative computer tomography
QUS	Quantitative ultrasound
SAS	Statistical Analysis Software
SD	Standard deviation
SI	Bone stiffness
SOS	Speed of sound
vBMD	Volumetric bone mineral density
WHO	World Health Organization
25OHD	25-hydroxyvitamin D

Summary

Although 90% of the maximal bone mass and bone strength are defined during the first two decades of life, bone health in children is usually investigated only if fractures or bone-related diseases have occurred. Monitoring and promotion of skeletal development, for instance by physical activity (PA) in early life could help to optimize peak bone mass and strength during growth in order to delay age-related bone loss and prevent osteoporotic fractures. However, bone health is usually assessed using x-ray imaging technologies, which are known to be expensive methods and to measure with ionized radiation. These disadvantages made it difficult for researchers to examine skeletal development in sufficiently large study samples over several years. Consequently, little is known about the dose-response relationship between PA and bone accrual and on the sustainable effect of PA on bone health in later life.

The assessment of calcaneal bone stiffness index (SI) by quantitative ultrasound technology (QUS) may be an alternative method to assess bone health in children in clinical settings and epidemiological studies. Compared to x-ray imaging technologies, QUS is radiation-free, cost-efficient and easy to apply. Besides those imaging technologies, the examination of bone turnover markers, such as serum carboxy-terminal telopeptide of type I collagen (CTX) or nutritional biomarkers such as calcium or vitamin D (25OHD) can also help to identify impaired bone metabolism at an early stage. However, the application of QUS as well as the range and interpretation of SI and CTX values in healthy children are still poorly investigated.

This thesis comprises two main goals. Firstly, this work aims to enhance knowledge on the age-specific distribution of SI, assessed by QUS (Achilles Lunar Insight) and serum CTX in 2-10-year-old healthy children. Secondly, the thesis aims to investigate the role of PA behaviour and nutritional biomarkers on SI in early life from an epidemiological perspective based on data of the European population-based multi-centre IDEFICS study (Identification and prevention of dietary- and lifestyle- induced health effects in children and infants).

The aims of this thesis were investigated and discussed in one narrative review and three original peer-reviewed publications.

In a first step, the reliability of SI measurements was investigated in children and adults. The results revealed higher precision errors for SI measurements in children (root-mean-squared coefficient of variation, CV_{RMS} = 7.2% - 9.2%) compared to adults (CV_{RMS} = 1.5% - 2.2%). Furthermore, low to moderate deviations of the SI values (0-5 units) between different QUS devices from the IDEFICS study were observed. The high precision errors of SI

measurements in children and the deviation of SI values between the devices may be partly explained by wrong positioning of the foot in the QUS device.

With regard to the first aim of this thesis, percentile values according to age, sex and height were established for SI and CTX on the basis of more than 10,000 2-10-year-old children. These reference values indicate a decline in SI with age and height in children until the age of 5-6 years, while an increase in SI has been observed from the age of about six years onwards. In contrast, the CTX percentile values show a linear positive association with age and height. These reference values are published and can be used to compare bone health of children in further epidemiological studies and clinical settings with a healthy reference population *(Herrmann D, et al., Int J Obes (Lond). 2014;38 Suppl 2:76-85).*

In view of the second aim, the current state of research on the association of PA with bone health was firstly summarized in a review that is based on observational and intervention studies, which have mainly assessed bone health by x-ray methods *(Herrmann D, et al., Bundesgesundheitsblatt Gesundheitsforschung Gesundheitsschutz. 2012;55(1):35-54).*

Subsequently, the association of PA on SI was further investigated in a cross-sectional as well as a case-control study in the course of the IDEFICS project. The cross-sectional results suggest that an increase of 10 minutes in vigorous PA per day or the participation in weight-bearing exercises may increase the SI by 2% - 3%. In contrast, light PA or sedentary behaviour was found to be weakly or negatively associated with SI *(Herrmann D, et al., submitted to Int J Behav Nutr Phys Act).* In the case-control study, the osteogenic effect of high-impact PA was further confirmed. However, it suggests that high-impact PA combined with high levels of calcium or 25OHD appears to be most beneficial for SI, while the observed single effect of calcium and 25OHD on SI appeared to be negligible *(Herrmann D, et al., Bone. 2015;78:142-149).*

The major strength of this thesis is the large sample of healthy European children with standardized data for SI, biochemical markers, anthropometric measures, medical history and different lifestyle behaviours of each child. These data were especially important for the derivation of the reference values on SI and CTX and the analysis of the case-control study.

However, the lack of longitudinal analyses for this thesis limits the interpretation of the findings with respect to causality and dose-response relationships between PA and SI. The fact that there were no data on fracture history in the IDEFICS children, as recommended by the International Society of Clinical Densitometry, is a further limitation with respect to the evaluation of the true value of a poor or pathological SI.

In conclusion, this thesis adds paediatric reference data on SI and CTX that form the basis for comparing bone health of children in further epidemiological studies and clinical settings with a healthy reference population. Furthermore, the comparable results of the original analyses in this work with findings from the reviewed studies, which assessed bone health with x-ray methods, support the use of QUS in future epidemiological and clinical studies for monitoring bone health during interventions in children. However, the reliability and comparability of the SI measurements with the same or with different devices must be taken into account, especially when applied in children.

In the light of the reported limitations, clinical trials that include children with bone-related diseases would be desirable in order to investigate the usefulness of SI measurements for diagnosis of paediatric osteoporosis. Furthermore, longitudinal studies are needed to fill the gap of knowledge on the sustainable effect of high-impact PA on bone health, and to fully understand the factors explaining variation of SI in children. Longitudinal QUS data from the follow-up surveys of the IDEFICS and I.Family study (Investigating the determinants of food choice, lifestyle and health in European children, adolescents and their parents) enable the investigation of SI over a time span of up to six years, covering the transition phase from childhood into early adolescence. These analyses may give first evidence on whether SI values are suitable in terms of monitoring skeletal development and detecting sustainable osteogenic effects in relation to changes in PA behaviour.

This thesis is structured into seven chapters: *Chapter 1 and 2* introduce the important role of bone health in childhood and summarize the research aims of this work. *Chapter 3* gives basic insights into bone physiology and describes the importance of PA for bone health taking into account the findings from the cross-sectional and case-control analyses of the IDEFICS study. While *Chapter 4* provides an outline on the current state of the assessment of bone health, *Chapter 5* describes the reliability study on SI measurements, which was conducted using different devices from the IDEFICS study. *Chapter 6* summarizes the strengths and limitations of this work. Finally, a conclusion of the thesis and ideas for future research are presented in *Chapter 7*.

Zusammenfassung

Knochenfestigkeit und ihre Assoziation mit dem Bewegungsverhalten bei Kindern. Eine epidemiologische Perspektive

Obwohl 90% der maximalen Knochenmasse und -festigkeit bereits in den ersten beiden Lebensjahrzehnten festgelegt werden, wird die Knochengesundheit bei Kindern in der Regel erst beim Auftreten von Frakturen oder Knochenerkrankungen untersucht. Die Beobachtung und Förderung einer optimalen Knochenentwicklung, zum Beispiel durch körperliche Aktivität (engl. physical activity, PA) im Kindesalter, könnte zur Optimierung der maximale Knochenmasse und -festigkeit beitragen und somit altersbedingten Knochenabbau verzögern sowie osteoporotischen Frakturen vorbeugen. Die Knochengesundheit wird üblicherweise mit bildgebenden Verfahren untersucht, die auf Röntgenstrahlung basieren und zudem teuer sind. Diese Nachteile erschweren die kontinuierliche Begleitung und Untersuchung der Knochenentwicklung in ausreichend großen Studienpopulationen über mehrere Jahre. Daher besteht ein Forschungsmangel zur Dosis-Wirkungsbeziehung zwischen PA und Knochenzuwachs sowie zum nachhaltigen Effekt von PA auf die spätere Knochengesundheit.

Die Messung des Knochensteifigkeits-Index (SI) mit quantitativen Ultraschallverfahren (QUS) am Fersenknochen könnte eine alternative Methode zur Untersuchung der Knochengesundheit bei Kindern in klinischer Praxis und epidemiologischen Studien sein. Im Vergleich zu Röntgenmethoden ist QUS strahlungsfrei, kostengünstig und leicht anwendbar. Frühzeitige Hinweise auf Störungen des Knochenstoffwechsels können neben den bildgebenden Verfahren auch Untersuchungen bestimmter Knochenmarker, wie z.B. Serum Carboxy-terminales Telopeptid des Type I Kollagens (CTX) oder Nährstoffe wie Kalzium oder Vitamin D (25OHD) geben. Allerdings sind die Anwendung von QUS sowie die Verteilung und Interpretation von SI und CTX bei gesunden Kindern bisher wenig untersucht.

Diese Dissertation umfasst zwei Hauptziele. Zum einen soll der Forschungsstand über die altersspezifische Verteilung sowohl vom SI, gemessen mit QUS (Achilles Lunar Insight), als auch von CTX von 2-10-jährigen gesunden Kindern verbessert werden. Zum anderen werden die Zusammenhänge von PA und ernährungsbezogenen Biomarkern mit dem SI im Kindesalter in einer epidemiologischen Perspektive untersucht. Grundlage für die Analysen bilden Daten aus der europäischen multizentrischen IDEFICS-Studie (Identification and prevention of dietary- and lifestyle- induced health effects in children and infants).

Die Schwerpunkte der Dissertation wurden in einem narrativen Überblicksartikel und drei Originalartikeln (peer-reviewed) untersucht und diskutiert.

In einem ersten Schritt wurde die Zuverlässigkeit der SI-Messungen bei Kindern und Erwachsenen überprüft. Die Ergebnisse zeigten höhere Präzisionsfehler der SI-Messungen bei Kindern (‚root-mean-squared' Variationskoeffizient, CV_{RMS} = 7.2% - 9.2%) im Vergleich zu Erwachsenen (CV_{RMS} = 1.5% - 2.2%). Des Weiteren wurde zwischen den verschiedenen QUS Geräten aus der IDEFICS-Studie eine geringe bis mäßige Variation im SI (0-5 Einheiten) beobachtet. Der höhere Präzisionsfehler der SI-Messung bei Kindern sowie die Abweichung der SI-Werte zwischen den QUS Geräten können teilweise durch eine falsche Positionierung des Fußes im Gerät erklärt werden.

Im Hinblick auf das erste Ziel der Dissertation wurden Perzentile nach Alter, Geschlecht und Größe für SI und CTX auf der Basis von mehr als 10,000 2-10-jährigen Kindern berechnet. Diese Referenzwerte deuten bei Kindern bis zum Alter von 5-6 Jahren auf eine Abnahme des SI mit zunehmendem Alter und Größe hin, während ab dem sechsten Lebensjahr ein Anstieg des SI zu beobachten ist. Demgegenüber zeigen die CTX-Werte eine lineare positive Assoziation mit zunehmendem Alter und Größe. Die veröffentlichten Referenzwerte können in anderen epidemiologischen Studien und in klinischer Praxis zum Vergleich der Knochengesundheit von Kindern mit einer gesunden Referenzpopulation verwendet werden *(Herrmann D, et al., Int J Obes (Lond). 2014;38 Suppl 2:76-85)*.

Hinsichtlich des zweiten Ziels wurde zuerst der aktuelle Forschungsstand über die Assoziation der PA mit der Knochengesundheit in einem Überblicksartikel zusammengefasst. Dieser basiert auf Beobachtungs- und Interventionsstudien, die hauptsächlich die Knochengesundheit mit Röntgenmethoden untersuchten *(Herrmann D, et al., Bundesgesundheitsblatt Gesundheitsforschung Gesundheitsschutz. 2012;55(1):35-54).*

Anschließend wurde im Rahmen des IDEFICS-Projekts die Assoziation von PA mit dem SI in einer Querschnitts- und einer Fall-Kontroll-Studie überprüft. Die Ergebnisse der Querschnittsanalyse deuten darauf hin, dass eine Erhöhung von starker PA um 10 Minuten pro Tag oder die Durchführung gewichtstragender Sportarten (engl. weight-bearing exercises) den SI um 2-3% erhöhen. Im Gegensatz dazu sind leichte PA oder sitzendes Verhalten schwach oder negativ mit dem SI assoziiert *(Herrmann D, et al., submitted to Int J Behav Nutr Phys Act).* Die Fall-Kontroll-Studie bestätigt diese osteogene Wirkung von stark belastender PA. Jedoch weist sie darauf hin, dass stark belastende PA kombiniert mit hohen Kalzium- oder 25OHD-Spiegeln am besten für einen optimalen SI sind. Demgegenüber

scheint der separate osteogene Zusammenhang von Kalzium und 25OHD mit SI unbedeutend zu sein *(Herrmann D, et al., Bone. 2015;78:142-149).*

Eine wesentliche Stärke der Dissertation ist die große Stichprobe von gesunden europäischen Kindern mit standardisierten Daten für SI, biochemische Marker und anthropometrische Maße sowie zur Krankheitsgeschichte und verschiedenen Lebensweisen des Kindes. Diese Daten waren besonders für die Ableitung der Referenzwerte des SI und CTX sowie für die Analyse der Fall-Kontroll-Studie von Bedeutung.

Da keine Längsschnittanalysen vorliegen, ist in dieser Arbeit die Interpretation der Ergebnisse in Bezug auf Kausalität und Dosis-Wirkungsbeziehungen zwischen KA und SI beschränkt. Eine weitere Einschränkung war die Tatsache, dass keine vergangenen Frakturen des Kindes erfasst wurden, wie es die ‚International Society of Clinical Densitometry' empfiehlt. Das Wissen darüber kann besseren Aufschluss über schlechte oder pathologische SI-Werte geben.

Zusammenfassend stellt diese Dissertation pädiatrische Referenzwerte für SI und CTX zur Verfügung, die in weiteren epidemiologischen Studien und in klinischer Praxis eine Grundlage für den Vergleich der Knochengesundheit von Kindern mit einer gesunden Referenzpopulation bilden können. Darüber hinaus unterstützen die vergleichbaren Ergebnisse aus dieser Arbeit mit den zusammengefassten Ergebnissen der Studien im Übersichtsartikel den Einsatz von QUS in weiteren epidemiologischen und klinischen Studien, um die Knochengesundheit während Interventionen zu beobachten. Jedoch muss besonders bei Kindern die Zuverlässigkeit der SI-Messungen und die Vergleichbarkeit verschiedener eingesetzter Geräte berücksichtigt werden.

Angesichts der beschriebenen Einschränkungen sind klinische Studien von Kindern mit knochen-assoziierten Erkrankungen wünschenswert, um den Aussagewert des SI für die Diagnose von kindlicher Osteoporose zu überprüfen. Darüber hinaus sind Längsschnittstudien erforderlich, um die Wissenslücke über die nachhaltige Wirkung von stark belastender PA auf die Knochengesundheit zu schließen und um die Faktoren, die die Variation des SI bei Kindern beeinflussen, vollständig zu verstehen. Längsschnittdaten von QUS-Messungen aus den Folgeuntersuchungen der IDEFICS- und der I.Family-Studie (Investigating the determinants of food choice, lifestyle and health in European children, adolescents and their parents) ermöglichen die Untersuchung der SI-Entwicklung unter Berücksichtigung des Übergangs vom Kindes- ins Jugendalter über sechs Jahre. Diese Analysen könnten erste Hinweise darauf geben, ob sich der SI für die Überwachung der Knochenentwicklung im

Kindesalter sowie für die Erkennung von kleinen Abweichungen im Knochen nach Veränderungen im Bewegungsverhalten eignet.

Die Dissertation ist in sieben Kapitel gegliedert: *Kapitel 1 und 2* leiten mit der Bedeutung der Knochengesundheit im Kindesalter ein und fassen die Forschungsziele dieser Arbeit zusammen. *Kapitel 3* enthält grundlegende Einblicke in die Physiologie des Knochens und beschreibt die Bedeutung von PA für die Knochengesundheit unter Berücksichtigung der Erkenntnisse aus den Querschnitts- und Fall-Kontroll-Analysen der IDEFICS-Studie. Während *Kapitel 4* einen Überblick über den aktuellen Forschungsstand zu Erfassungsmethoden der Knochengesundheit gibt, beschreibt *Kapitel 5* die Reliabilitätsstudie zu den SI-Messungen, die mit den QUS-Geräten aus der IDEFICS-Studie durchgeführt wurde. *Kapitel 6* fasst die Stärken und Grenzen der Arbeit zusammen. Abschließend werden im *Kapitel 7* Schlussfolgerungen aus der Arbeit gezogen sowie zukünftige Forschungsideen vorgestellt.

1 Introduction

"Osteoporosis: Pediatric disease with geriatric consequences"

Linda Hightower (2000), Orthopaedic Nurses and Facilitator of the Greater Missoula Area Osteoporosis Support Group.[1]

This publication title of Hightower is increasingly a common view of many researchers in the field of bone development and osteoporosis. However, most people usually pay attention to their bone health when it is already too late, that is, when osteoporosis or osteoporotic fractures have already occurred. The problem is that, once bone mass is degraded in adulthood, it can hardly be re-established again.

Skeletal fragility usually occurs due to aging, a lack of mechanical loadings or several chronic bone-related diseases. These diseases can inhibit bone formation, increase bone resorption or reduce the responsiveness of bone to mechanical loads, leading to an increased risk for osteoporosis.[2,3] The World Health Organisation (WHO) defines osteoporosis as a "skeletal disease characterized by low bone mass and microarchitectural deterioration of bone tissue with a consequent increase in bone fragility and susceptibility to fracture" (WHO Technical Report, 2007, p. 53).[4] The WHO reported that 75 million people in the United States, Europe, and Japan are affected by osteoporosis that is strongly associated with an increased morbidity and mortality. Due to demographic changes and an aging population it is predicted that the prevalence of osteoporosis and osteoporotic fractures will increase during the next decades.[4,5]

Several researchers discuss the acquisition of an optimal peak bone mass as an essential factor in determining the onset of osteoporosis and fractures.[6-8] Peak bone mass is "the amount of bone present in the skeleton at the end of its maturation process" (Rizzoli et al., 2010, p. 295).[9] Thus, the higher the achieved peak bone mass, the more an individual is able to lose bone mass in older age.[10] Some researchers argue to focus on peak bone density, since bone density captures microarchitectural bone characteristics and thus indicates its strength, which may be of importance in terms of fracture risk. Based on a longitudinal simulation of age-related bone loss, Hernandez et al. predicted that a 10% increase in peak bone mineral density delays the development of osteoporosis by 13 years.[6] Although, both, peak bone mass and density seem to play an important role for bone health over the life course, most research has only considered on peak bone mass. The age at which peak bone mass is reached varies individually, by sex and between skeletal regions. It is mostly achieved during the late second and early third decade in life, during which about 90% of adult bone mass is acquired.[9,11-15]

Until peak bone mass is reached, modelling and remodelling processes are rapid and bone formation rates exceed bone resorption rates. After achieving peak bone mass, modelling and remodelling processes slow down, bone resorption becomes more predominant and age-related bone loss accelerates. This is how bones get porous and the risk for osteoporosis and fractures increases. The reduced levels of oestrogen in (post-) menopausal women lead to a renewed increase of the remodelling processes. This simultaneously means an accelerated bone resorption, leading to reduced mineral content.[2,7,11,16,17] This is mainly why women of this age have a higher risk of bone fragility and osteoporosis than men.[18] In view of this background, bone researchers and physicians are encouraged to pay more attention on investigating and monitoring bone health as well as osteoporosis prevention early in life.[7,15]

Skeletal development and bone health is largely determined by genetic factors that contribute up to 60% - 80% of the variance of bone characteristics.[9,15,19-21] However, modifiable lifestyle behaviours such as physical activity (PA), sedentary behaviour and diet seem to contribute to bone health already in early life. In particular, moderate-to-vigorous PA (MVPA) and weight-bearing exercises have been observed to sustainably affect skeletal development and bone health from childhood into adolescence.[22-25] Researchers assume that there exists a potential sustainable osteogenic effect of sufficient MVPA and weight-bearing exercises in childhood to delay age-related bone-loss and decrease osteoporotic fracture risk later in life.[9] However, there are no longitudinal studies over the life course investigating this issue. The lack of longitudinal studies is largely due to the large number of challenges in the assessment of bone health and PA, especially in young and growing individuals. This thesis primarily focuses on the difficulties in the assessment of bone health in children. The challenges faced in the assessment of PA were partly discussed in the manuscripts written in the context of this work.

In children, bone health is usually investigated only when traumatic and spontaneous fractures have occurred. This is mainly due to the concerns of the applied x-ray imaging techniques.[3,26] Thus, epidemiological research on bone health in apparently healthy children, especially in children below the age of five, is lacking. Alternative assessment methods such as quantitative ultrasound technologies (QUS) or osteogenic biochemical markers such as osteocalcin or carboxy-terminal telopeptide of type I collagen (β-crosslaps, CTX) for evaluating bone health and metabolism in children are of increasing interest to fill this research gap. However, they are still poorly investigated and validated. This hinders researchers to examine an optimal skeletal development in young individuals and to provide evidence in terms of an adequate dose of PA in early life in order to ensure healthy bones into old age.

2 Research Aims

This thesis aims to provide first data on the age distribution of bone stiffness (SI), assessed by QUS, and serum CTX as indicators of bone health in 2-10-year-old children. Furthermore, this thesis aims to investigate the role of PA behaviour on SI in children of this age group from an epidemiological perspective based on the European population-based multi-centre IDEFICS study (Identification and prevention of dietary- and lifestyle- induced health effects in children and infants).[27-29] In this study, calcaneal SI was measured with Achilles Lunar Insight (GE Healthcare, Milwaukee, WI, USA) in more than 10,000 children.[30]

In order to achieve the aims of this thesis, following objectives were investigated and discussed in one narrative review and three original peer-reviewed publications:

I. Review of previous observational and intervention studies to gather existing evidence on the impact of PA behaviour and exercise on skeletal development and bone health over the life course *(Article 1, see App. A)*.[22]

II. Investigation of the association of different PA intensities, such as light, moderate and vigorous PA, sedentary behaviour, and muscular fitness with SI in children *(Articles 2-3, see App. B-C)*.[31]

III. Investigation of nutritional biomarkers, which are related to skeletal development, such as calcium, vitamin D and phosphorus, and their modifying effect on the association of PA with SI in children *(Article 3, see App. C)*.[31]

IV. Provision of age-, sex- and height-specific percentile values and curves on SI and CTX in 2-10-year-old healthy children to determine the age trend of these bone parameters in childhood *(Article 4, see App. D)*.[30]

V. Investigation of the reliability of SI measurements using QUS devices from the IDEFICS study *(see Chapter 5)*.

The content of this thesis is hereafter described in Chapters 3-7. *Chapter 3* gives basic insights in the physiology and functioning of bone. In addition, this chapter refers to epidemiological investigations of the IDEFICS study, which are part of the objectives of this thesis. While *Chapter 4* provides an outline on how bone health can be assessed in children, adolescents and adults, *Chapter 5* describes the reliability of QUS measurements within one device and between the different devices, which were applied in the IDEFICS study. *Chapter 6* summarizes the strengths and limitations of this work. Finally, a conclusion of the

thesis and a summary of what we need to know from further epidemiological studies are presented in *Chapter 7*.

3 Physiology of Bone Development

3.1 Bone Biology

"Bone is a dynamic mineralised connective tissue" (Grabowski, 2009, p. 32).[32] Its structure is complex and yet not fully explored and understood. The skeleton has several physiological functions. It provides mechanical support and permits movement and locomotion. Furthermore, bones store several minerals such as calcium, magnesium or phosphate and simultaneously are responsible to maintain calcium-phosphate homeostasis. Finally, the skeleton protects the internal organs from physical damage.[32,33]

Macroscopically, bone consists of cortical (compact) and trabecular (cancellous) bone. The two forms are similar in their cellular composition but, due to their different functions, different in their structural properties. The cortical tissue provides the mechanical and protective function. The trabecular tissue is predominantly responsible for the metabolic and homeostatic purposes of bone. Thus, cortical bone is much denser, harder, stronger and stiffer, while trabecular bone is spongy and less dense.[2,34-36]

Bone growth basically occurs by bone modelling and remodelling. Bone modelling is characterized by formation processes while bone remodelling is characterized by the alternation of resorption and formation processes (= bone turnover).[37] Bone turnover processes are primarily determined by genetic factors, which have usually been investigated in animal studies, and which are poorly understood in humans.[38,39] For instance, the hypothalamic neuropeptide Neuromedin U was reported to influence bone formation in Neuromedin U-deficit mice that were observed with higher bone mass.[39] Analyses in the IDEFICS study demonstrated this phenomenon in children, where variant alleles of Neuromedin U were negatively associated with SI *(Article 5, see App. E)*.[21]

The matrix of both, cortical and trabecular bone, comprises organic and anorganic components. The organic part consists of approximately 90% - 95% type I collagen and the rest of non-collagen proteins such as osteocalcin.[32,40] In the bone matrix, mature bone cells (= osteocytes) are embedded and held together by type I collagens by crystals of calcium-phosphate hydroxyapatite (= anorganic components).[2] Osteocytes develop from osteoblasts during bone formation and are responsible for calcium homeostasis.[32,41] Osteoclasts are responsible for the resorption of the bone matrix.[2,32]

Synthesis products of osteoblasts are proteins such as osteocalcin, bone alkaline phosphatase (BAP) or procollagen type I N- / C-propeptide (PINP / PICP). They are components in the formation of the bone matrix (= formation biomarkers). During the resorption of the bone matrix by osteoclasts, type I collagen and its telopeptide are fragmented into CTX in urine and serum, or respectively N-telopeptide in urine (NTX) (= resorption biomarkers). In addition, bone minerals such as calcium and phosphate are released into the blood circulation. In formation and resorption markers, the 'N-' and 'C-' terminus refers to the beginning (= amine terminus or N-terminal) and ending (= carboxyl terminus or C-terminal) of the amino acid chain of the protein. Bone formation and resorption biomarkers are used as a diagnostic tool for bone formation and resorption, where the combination of both indicates bone turnover *(see Chapter 4.4)*.[10,40,42,43]

For the mineralization of the unmineralized bone matrix by calcium-phosphate hydroxyapatite, the vitamin D synthesized 25-hydroxyvitamin D (25OHD) plays an important role. Its biologically active form 1,25-dihydroxyvitamin D stimulates the intestinal absorption of calcium and phosphorus, and it promotes the differentiation of osteoblasts.[33]

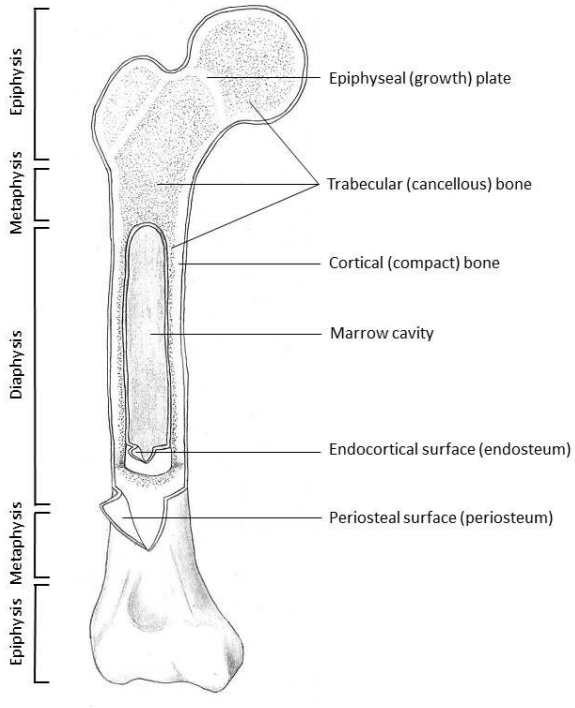

Figure 1 Structure of a femur of a child
Adapted from Henry Gray, *Anatomy of the Human Body* (1918). Source: http://www.bartleby.com/107/illus244.html

However, to investigate bone health, most epidemiological studies examined long bones, such as the femur or tibia in the lower limbs, or the radius and ulna in the upper limbs using imaging technologies *(see Chapter 4.1)*. Figure 1 shows the structure of a long bone. Long bones predominantly consist of cortical tissue that surrounds the inner smaller trabecular regions and marrow cavity on the diaphysis. The cortical bone is surrounded by the periosteum in which modelling processes that induce bone formation take place. This results in an increase in bone mass and size.

The endosteum lies between the trabecular surface and marrow cavity, where remodelling takes place. The metaphyseal and epiphyseal sites of long bone consists of more trabecular tissue, where longitudinal bone growth occurs.[2,32] Due to its spongy surface, trabecular bone has an 8-fold greater surface area accessible to osteoclastic resorption per unit volume. Thus, it undergoes faster remodelling and more rapid changes in bone mass and structure than cortical bone.[34] Therefore, the assessment of the skeletal sites consisting of trabecular bone, such as the femoral neck or calcaneus, is of particular interest when monitoring bone health, especially during bone-related treatments and interventions.

3.2 The 'Mechanical Competence' of Bone

The main function of bone is to provide support for mechanical loadings which occur during PA. In its functionality, healthy bones require both, some stability, but also a certain bending, torsional and compressive strength.[7,32,44] Bone researchers mostly define the function of bone as its 'mechanical competence', which is not merely dependent on sufficient bone mass but also on sufficient bone strength.[32,45] The strength of a bone is determined by its structural properties, including its mass, material composition, architecture and density. Bone must be stiff enough to resist mechanical loadings from PA or exercise and must also be flexible enough to absorb the energy from these loads and be able to change or adapt its bone structural properties without cracking.[2,36]

The largest loads on bone are produced by muscular activity.[46-48] Harold Frost, one of the most important scientists in the field of bone physiology, described the close interaction between bone and muscles as follows: "[…] muscles must overcome the resistance of body weight multiplied by the bad lever arms against which most muscles work. For that reason it takes well over 2 kg of muscle force on bones to move each kilogram of body weight […]" (Frost, 1999, p. 99).[49] Such mechanical loads from muscular activity, and thus PA, cause bone modelling on the cortical and remodelling on the trabeculae tissue to increase bone mass and strength, respectively.[2,45,48,49] Consequently, the absence of mechanical loads due to inactivity leads to bone loss, which has been observed in bedridden patients.[50] Frost described this biological mechanism of bone growth and adaption in his 'mechanostat theory'. His theory indicates the dominant role of mechanical loads on bones that control modelling and remodelling processes by up to 40%. Here, the responsiveness of osteoblasts, osteocytes, and osteoclasts to mechanical loads is genetically determined by lower and upper thresholds that initiate or inhibit bone modelling and remodelling. Mechanical loadings need to exceed a

certain threshold to cause small microcracks. Thus, the bone is automatically forced to adapt its strength and structure to prevent further peak loads from causing spontaneous fractures. If the thresholds are not reached, modelling and remodelling processes are not activated. Frost compares the mechanostat of bone with an air-conditioning system in a house. The system turns on only when the temperature exceeds the thermostat setting, and stays off at lower temperatures.[49] Non-mechanical factors, such as hormones, nutrients or drugs determine only about 3% - 10% of bone's strength and structure. They can help or hinder the influence of mechanical loads; but they cannot replace it.[45,49]

According to this biological background, bone health is not only determined by genetic factors but also by mechanical loadings due to muscles, PA and exercise that force the bone to renew and adapt its structure, strength and composition. The knowledge of this 'mechanical competence' of bone is of relevance in terms of developing intervention strategies for optimizing peak bone mass.

3.3 Epidemiological Investigations

Based on observational and intervention studies, the review on the *"Impact of physical activity and exercise on bone health in the life course"* concludes that, especially MVPA and school-based exercise programmes, consisting of weight-bearing exercises such as jumping, seem to be most beneficial for bone accrual in children and adolescents. The researchers explain the beneficial osteogenic effect of weight-bearing exercises due to the short-impact durations of acute bouts and the high ground reaction force that induce muscle contractions and force the bone to adapt.[51,52] These epidemiological observations underpin Frost's theory that a certain threshold of mechanical loads due to muscular activity, and thus due to exercise, needs to be exceeded, so that the bone can be strengthened. In contrast, during adulthood, the sensitivity of bone to PA seemed to decrease. Two limitations of the reviewed studies were the assessment of bone status with x-ray imaging technologies *(see Chapter 4.2)* and the small sample sizes *(Article 1, see App. A)*.[22] Cross-sectional analyses of the IDEFICS data in over 4.500 2-10-year-old children confirmed the strong association of high-impact PA, such as weight-bearing exercises in a sports club and habitual MVPA, with SI. In contrast, light PA does not seem to have an effect on SI. These results were comparable in boys and girls in this age group. Furthermore, it was observed that the adverse impact of sedentary behaviour on bone health can be partly counteracted by high-impact PA in children and adolescents *(Articles 2 and 3, see App. B and C)*.[31,53-55]

According to Frost et al., non-mechanical factors such as nutrients are influencing bone accrual to a less extent than PA.[45,49] A case-control study embedded in the IDEFICS study ($N_{cases} = 603$, $N_{controls} = 1{,}216$) confirmed this hypothesis. The results were not statistically significant and indicated weak odds for a low SI (<15th age-, sex- and height-specific SI percentile value) in children in the first tertile of serum calcium (<2.5 mmol/l) or 25OHD (<34.9 nmol/l) compared to children in the upper tertile. However, it should be noted, that values of serum calcium varied within normal ranges and more than 72% of the cases and controls were above the threshold of vitamin D deficiency (≥30 nmol/l).[31,56] Thus, it can be considered unlikely to observe a significant impact on SI. Nevertheless, the results of the case-control study imply that children with lower calcium or 25OHD levels who spent less than 37 minutes per day in MVPA or performed no weight-bearing exercises had a nearly 2-fold increased risk for lower SI compared to their controls. In other words, high-impact PA combined with high levels of calcium or 25OHD appears to be most beneficial for SI *(Article 3, see App. C)*.[31]

The positive influence of muscular mass and strength on bone health in children was confirmed in numerous epidemiological studies, including the IDEFICS study *(Article 2, see App. B)*.[57-60] Consequently previous studies reported on an adverse effect of high body fat mass on bone strength in early life, that was attributed to low PA *(Article 1, see App. A)*.[57,61-63] A recent publication of the IDEFICS study confirmed the positive association of fat-free mass, as a proxy for muscular mass, as well as the negative association of fat mass with SI in 2-10-year-old children *(Article 6, see App. F)*.[64] Nevertheless, the stratified analysis of preschool (2-<6 years) and school children (6-10 years) indicates a positive association of fat-free mass with SI only in school but not in preschool children. This may be due to the higher proportion of organ tissues (than muscular mass) in younger children which contributes to fat-free mass *(Article 2, see App. B)*. Furthermore, fat-free mass was calculated including height. The age-, sex- and height-specific reference values implicate a similar negative trend of SI with increasing height in preschool children, while a positive association of height with SI was observed in school children *(Article 4, App. D)*.[30]

In summary, these epidemiological results support the relevance of mechanical loads due to high-impact or high intensities of PA, a high muscular fitness and reduced sedentary behaviour on bone accrual already in children. Furthermore, the results of the IDEFICS study on SI, assessed by QUS, are similar to results of previous studies that investigated bone health assessed by x-ray imaging technologies in small sample sizes.

4 Assessment of Bone Health

4.1 Diagnosing Osteoporosis and Fracture Risk

The International Society of Clinical Densitometry (ISCD) publishes official position papers on recommendations for the clinical application of assessment methods for bone health in children, adolescents and adults.[65] Dual-Energy X-ray Absorptiometry (DXA) is the most widely used method for diagnosing osteoporosis and predicting fracture risk in clinical settings and for evaluating bone health in epidemiological studies.[3] It measures the bone mineral content (BMC) and the areal bone mineral density (aBMD). The hip (including femoral neck), spine and forearm are the most common skeletal sites of fractures in the elderly. In adults aged 20 years and older, osteoporosis is diagnosed by comparing the individual BMC/BMD with the average BMC/BMD of a reference population of healthy premenopausal women, in whom peak bone mass is assumed. The comparison of an individual value such as BMC or BMD to the average value of healthy young reference population is defined as T-score. This reference population for BMC and BMD is based on 20-29-year-old healthy women from the National Health and Nutrition Examination Survey reference database (NHANES).[4,66]

The T-score is the only diagnostic criteria for osteoporosis in adults and describes the deviation of the individual BMC/BMD from the average BMC/BMD of the reference population by using standard deviations (SD). The WHO defined following operational criteria for diagnosing osteoporosis: The deviation of the T-score (i.e. of an individual BMC/BMD) by 2.5 SD or more below the average BMC/BMD (i.e. T-score ≤ -2.5) is defined as osteoporosis. A milder form of bone fragility is osteopenia which is defined as a T-score between 1 and 2.5 SD below the young adult female average value (T-score <-1 to -2.5, see Table 1).[5,7,67]

Osteoporosis and osteoporotic fractures in children are uncommon and their prevalence is poorly investigated. Paediatric osteoporosis is usually based on either genetically determined diseases (= primary osteoporosis) or on endocrine and chronic diseases (= secondary osteoporosis, see Table 2). Here, metabolic changes, the reduced ability to be physically active, malnutrition, malabsorption or medical treatments inhibit bone formation or increase bone resorption, and thus increase fracture risk.[3,68,69]

Table 1 Definition of diagnosing osteopenia and osteoporosis in children, adolescents, adults and elderly, based on the standard deviation (SD) of bone mineral content or bone mineral density measured with dual-energy x-ray densitometry.

	Children and adolescents[1] 5-19 years	**Adults and elderly[2] ≥20 years**
Osteopenia		1 to 2.5 SD below the mean value for healthy premenopausal women (T-score < -1 and ≥ -2.5)
Osteoporosis	1) Presence of a clinically significant fracture history, that is, one or more of following fractures: - Long bone fracture of the lower extremities; - Vertebral compression fracture; - Two or more long-bone fractures of the upper extremities AND 2) 2 SD or more below the healthy reference group of children in the same age, sex and body height (Z-score ≤ -2)	-2.5 SD or more below the mean value for healthy premenopausal women (T-score ≤ -2.5)

[1] Criteria based on the International Society of Clinical Densitometry (ISCD)[3]
[2] Criteria based on the World Health Organization (WHO)[5,67]

The WHO diagnostic criteria for osteoporosis in adults, i.e. the T-score, should not be applied in children who have not yet reached their peak bone mass. Age and growth need to be taken into account when comparing bone health of a child with a healthy child reference population. Here, the Z-score can be used to compare an individual value with an age-matched or rather age- and height-matched reference population.[7] This was considered for deriving the age-, sex- and height-specific reference values on SI and CTX in 2-10-year-old IDEFICS children *(Article 4, see App. 4)*.[30]

The predictive value of BMC and BMD for skeletal fragility in children is not well investigated. Therefore, according to the International Society of Clinical Densitometry (ISCD) the diagnosis of osteoporosis in children should not be made only by DXA measurements. It also requires the assessment of the fracture history of the child. Thus, the ISCD defines paediatric osteoporosis in 5-19-year-old "as low BMC/BMD in the presence of a clinically significant fracture history" (Bianchi et al., 2010, p. 39). A fracture history is clinically significant when the child suffered of either one or more of the following fractures: "a long bone fracture of the lower extremities, or a vertebral compression fracture, or two or more long-bone fractures of the upper extremities" (Bianchi et al., 2010, p. 39). "Low bone mineral content or bone mineral density is defined as a BMC or areal BMD Z-score that is

Table 2 Diseases considered by the Position Development Conference as potentially affecting the skeleton and associated with an increased fracture risk in children and adolescents (adopted and amended according to the International Society for Clinical Densitometry (ISCD) in Bianchi et al., 2010)[3]

Diseases that potentially affect the skeleton	
Primary Bone Disorders	Idiopathic juvenile osteoporosis
	Osteogenesis imperfecta
Secondary Bone Disorders	Inflammatory diseases
	Inflammatory bowel disease
	Juvenile idiopathic arthritis
	Cystic fibrosis
	Chronic immobilization
	Cerebral palsy
	Myopathic disease
	Epidermolysis bullosa
	Endocrine disorders
	Turner syndrome
	Anorexia nervosa
	Hyperthroidism, Hyperparathyroidism
	Cushing's syndrome
	Renal failure disease
	Juvenile diabetes
	Idiopahthic hypercalciuria
	Liver disease/failure
	Cancer and therapies
	Acute lymphocytic leukemia
	Transplant bone disease
	Hematologic disorders
	Thalassemia
	Other
	Immobilization
	Radiation therapy
	Rheumatoid arthritis

less than or equal to -2.0, adjusted for age, gender and body size, as appropriate" (Bianchi et al., 2010, p. 42).[3] In other words, for diagnosing paediatric osteoporosis, a DXA measurement in children should be only performed, when the child has a clinical significant fracture history (Table 1). The clinical relevance of the fracture assessment is that, traumatic fractures in healthy children are not common and were mainly observed in children who already had low BMD, who performed competitive sports, or who were involved in an accident. Furthermore, any fracture before the age of five years increases the risk of subsequent fractures 2- to 3-fold during growth.[70] In longitudinal and retrospective studies, pre- and peripubertal children with prior fractures had lower BMC accrual and where observed with lower trabecular BMD at the metaphyseal site of femur and tibia compared to those who were fracture-free.[71,72]

Next to the assessment of fracture history, several individual predispositions and lifestyle risk factors should be considered before conducting DXA in a paediatric population. There is evidence that pre- and perinatal determinants such as preterm birth, born small-for-gestational-age or in general low birth weight result in a decreased bone accreation.[73-76] A cross-sectional study in 25-year-old women demonstrated the association of low birth weight with a low BMC that, according to the researchers, consequently adversely affected the attainment of peak bone mass.[77] However, based on the IDEFICS data, no association between birth weight and SI at the ages 3 to 11 was observed *(Article 7, see App. G)*.[78] Commonly reported lifestyle risk factors are physical inactivity or competitive sports, an inadequate diet such as insufficient calcium intake, the avoidance of milk products, consumption of carbonated beverages or anorexia. For the detection of insufficient bone-related minerals in time, the assessment of calcium, vitamin D and phosphate levels in serum may be of relevance.[9,69,70,79] However, with regard to the results of the case-control study, reported in *Chapter 3.3*, calcium, 25OHD and phosphate levels in normal ranges appear to be only weakly associated with low SI *(Article 3, see App. C)*. Finally, the intake of medications or medical treatments, which may adversely affect bone health and thus increase fracture risk, needs to be considered.[31,69,70]

4.2 X-ray Imaging Technologies

Although DXA is used as the gold standard for diagnosing osteoporosis and predicting fracture risk in adults, it provides only little information on bone strength, geometry and microarchitecture. These bone characteristics may be meaningful in terms of evaluating the 'mechanical competence' and thus fracture risk of bone.[80,81] The two-dimensional image projection of aBMD does not indicate true bone density. True density and strength of bone depends on BMC and its depth and size, that is, the volume of the bone. This especially plays an important role in growing individuals, where aBMD may result in underestimation of bone density in short individuals and an overestimation in tall individuals.[82,83] Furthermore, DXA does not distinguish between cortical and trabecular bone.

A more sophisticated method to assess structural bone parameters is the peripheral quantitative computer tomography (pQCT). This procedure assesses, amongst other structural parameters, the three-dimensional structure of bone by volumetric BMD (vBMD, g/cm^3) that is less biased by bone size and thus growth. Furthermore, it distinguishes between cortical and trabecular bone, which may be of relevance in monitoring the progression of bone-related

diseases or medical treatments.[69] Both types of bone tissue have been reported to respond differently to medications or PA. For instance, children with chronic kidney disease are characterized by increased trabecular bone mass but reduced cortical bone mass.[3,83] Nevertheless, there is weak evidence in terms of the effect of PA on cortical and trabecular bone. In a cross-sectional study of 10-13-year-old girls (N=60), trabecular bone seemed to be affected only by high (average metabolic equivalent of task, MET value = 46.9 hours/day) but not by moderate PA levels (average MET = 39.1 hours/day).[84] An intervention trial consisting of a 12-week jumping program in 54 3-18-years old children reported an increase in trabecular BMC only in pubertal children. In contrast, in pre- and peripubertal children they observed an effect on cortical bone with no apparent increase in trabecular bone.[85]

However, pQCT also has disadvantages, in particular with respect to the application in observational studies. There are only few epidemiological studies that have used pQCT and which included small sample sizes.[84,85] This may be explained, among other, by the exposure to ionized radiation, which is comparable with DXA (0.08-4.6 μSv).[44,86] Furthermore, the ISCD reported weak evidence on reference data for the clinical use of pQCT in order to predict fracture risk or diagnosing low bone mass in young individuals.[26]

4.3 Quantitative Ultrasound Technologies

The application of QUS technologies in epidemiological studies and clinical settings is an increasingly used alternative assessment method in order to evaluate fracture risk, skeletal development and to investigate the influence of certain risk and protective factors on bone health. QUS measurements are radiation-free, cost-efficient and portable.[87,88] This allows a better application of QUS in epidemiological studies, compared to DXA or pQCT, to examine bone health in larger paediatric samples.

There are various types of QUS devices available measuring different skeletal sites (e.g. phalanges, hand, heel, or tibia), with different frequencies of ultrasound, different pathways of ultrasound transmission or different QUS parameters.[66,87,88] Calcaneal QUS, as applied in the IDEFICS study, is the only validated skeletal site and approved by The Food and Drug Administration (FDA) for predicting the risk of hip fractures and for monitoring bone changes in postmenopausal women and men over 65 years.[89] It is used as a pre-screening test for DXA. Similarly, various calcaneal QUS devices are available (e.g. Achilles, SAHARA, CUBA, UBIS).[80,90] This makes comparison of QUS results difficult.

The clinical relevance to measure the calcaneus is that it consists of 90% trabecular bone, and it is similar to the bone structure in hip and spine, which are the most common skeletal sites of osteoporotic fractures.[80,88,90] Its high trabecular tissue, its weight-bearing skeletal site and thus its high remodelling and metabolic rate characterize the calcaneus to have the best structure responding to age-, disease- or therapy induced bone alterations than other skeletal sites.[91] However, this needs to be confirmed in further clinical intervention studies.

The mechanisms of QUS in assessing bone characteristics are still poorly understood. Roughly described, QUS provides information on bone structure and strength through traveling ultrasound waves.[88,92-94] Here, two parameters are measured: One parameter is the speed of ultrasound waves (SOS, m/s), that is influenced by bone structure and density. In several studies, reviewed by Bouxsein and Radloff, ultrasound velocity was described to characterize elastic properties of trabecular and cortical bone.[93] As a second parameter, QUS measures the broadband attenuation of ultrasound (BUA, dB/MHz). Here, a broadband ultrasonic impulse is sent through the bone. Its reduction in intensity at different frequencies is measured in comparison to a reference medium, such as water.[88,89,92] Validation studies showed that the combination of SOS and BUA were slightly better at predicting bone strength than either parameter alone.[93] Thus, for providing better precision of SOS and BUA, the SI is calculated as a percentage based on normalized and scaled values of SOS and BUA as $SI = (0.67 * BUA + 0.28 * SOS) – 420$.[89,95,96] In this work, the SI values are expressed as 'units' instead as percent in order to avoid confusion with other percentages, and which is consistent with previous studies.

Despite the approval of the FDA, the ISCD does not recommend using calcaneal QUS measurements for diagnosing osteoporosis or for monitoring skeletal changes during treatments in children and adults.[80,87,90] On the one hand, previous studies that compared QUS with DXA measurements in adults showed strong correlations of up to 0.9 between calcaneal SI and BMD at the calcaneus, hip or spine.[90,97] Furthermore, lower QUS values were observed in women with multiple risk factors (e.g. significant fracture history or intake of bone-affecting medications) or osteoporotic bones, compared to the healthy control groups.[87,90] On the other hand, in contrast to DXA, there are no established QUS diagnostic criteria for osteoporosis.[87,88,90] The application of the WHO DXA criterion (i.e. T-score $\leq$ -2.5) for calcaneal QUS measurements to identify individuals with osteoporosis showed low sensitivity and specificity. Only about 30% of postmenopausal women with osteoporosis, diagnosed by DXA, were also detected by QUS measurement.[80,87,90] Thus, the DXA T-score $\leq$ -2.5 for diagnosing osteoporosis should not be used when applying QUS. A referral criterion of QUS

for an additional DXA examination has been established for the application of QUS as a pre-screening test to identify women with osteoporosis. The ISCD recommends a QUS criterion (i.e. a T-score) for detecting osteoporosis with a sensitivity of 90%. In other words, the T-score threshold of QUS needs to detect 90% of women who have a DXA T-score $\leq$ -2.5.[80] Such a threshold needs to be device-specific and thus varies between different QUS devices.[90,98] For the Achilles device, as applied in the IDEFICS study, a T-score $\leq$ -1 (based on 20-35-year-old healthy women) was established as a criterion for the recommendation of subsequent DXA measurement.[89]

It however needs to be kept in mind that QUS and DXA measurements give different information on bone tissue and structure.[88] Thus, QUS does not replace DXA for diagnosing osteoporosis but may give additional information for fracture risk.

There is hardly any evidence on the validity of QUS parameters for predicting skeletal fragility in children. In an IDEFICS sub-study of 3-8-year-old children (N = 24) investigating the comparability between QUS and DXA measurements, no correlations were observed between calcaneal SI and aBMD of the whole body and spine.[99] The small sample size and the different examined skeletal sites by DXA and QUS in growing children may partly explain this result. Nevertheless, a number of studies with larger sample sizes that compared calcaneal SI (Achilles) with BMD (DXA) of whole body, lumbar spine and hip in 5-19-year-old children and adolescents reported correlation coefficients between 0.5-0.7.[96,100,101] A comparison of calcaneal SI with calcaneal BMD resulted in a correlation of 0.8 in 6-13-year-old children (N = 28).[96] In addition, reduced QUS measurements were observed in children with osteopenia, disturbance of growth or other bone-related disorders compared to their healthy control groups.[88,96]

Despite these comparison studies, QUS data from epidemiological studies for investigating bone growth or bone-related diseases in children are scarce. No official paediatric reference data are available for calcaneal QUS parameters to compare the bone status of a child with that of a healthy population. Only few normative data are published, usually expressing the SI values as a mean and SD, differentiated by age and sex. The values however do not take growth into account. This thesis, for the first time, provides age-, sex- and height-specific percentile values on SI, measured with Achilles Lunar Insight in 10,791 2-10-year-old European children who participated in the IDEFICS study. These reference data provide a unique possibility for the comparison of paediatric SI data collected in further epidemiological studies and may help to identify deviations from normal skeletal

development *(Article 4, see App. D)*.[30] However, there is still insufficient research on the validity and reliability of QUS in young age.[100,102-104] *Chapter 5* of this work gives an overview on the reliability of the applied QUS devices used in the IDEFICS study.

4.4 Osteogenic Biomarkers

Besides imaging technologies, the examination of biochemical formation and resorption markers in serum and urine can help to detect disturbances in bone metabolism or mineralization. The corresponding biomarkers can also be related to an increased fracture risk. An advantage of such biochemical markers is that changes, for example after interventions, occur already after 3-6 months, while changes in bone structure assessed by imaging technologies are only visible after 1-2 years.[70]

Several cohort and case-control studies have reported that reduced bone formation and normal or increased rates of bone resorption predict fracture risk in postmenopausal women.[42,105,106] The International Osteoporosis Foundation (IOF) and the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) recommend one bone formation marker (serum PINP) and one resorption marker (serum CTX) for use as reference markers for predicting fracture risk in adults.[42] The predicting value of these markers for fracture risk or an abnormal skeletal development in children has scarcely been investigated. Due to bone growth and higher bone remodelling, markers of bone turnover, and thus of bone formation and resorption, are higher in children compared to adults. However, similar to BMD or BMC, bone turnover markers in children are dependent on age, sex, growth and pubertal stage.[40,105]

5 Application of Quantitative Ultrasound in the IDEFICS Study

5.1 Standardized Measurements of Bone Stiffness

In this thesis, the analyses on SI are based on the IDEFICS cohort in which more than 18,000 children aged 2-11 from eight European countries (Sweden, Germany, Hungary, Italy, Cyprus, Spain, Belgium and Estonia) were examined. The main goal of this study is to investigate associations of biological and behavioural factors on lifestyle diseases in a longitudinal perspective. The baseline survey (T0) was conducted in 2007/08 and the first follow-up survey (T1) took place in 2009/10. The children from the IDEFICS study were re-examined in 2013/14 in a follow-up survey named I.Family (Investigating the determinants of food choice, lifestyle and health in European children, adolescents and their parents).

Difficulties in assessing bone health with x-ray imaging technologies in children, as described in *Chapter 4*, hinder researchers from identifying potential bone-related risk and protection factors on skeletal development during growth. The IDEFICS study is one of the first European studies to examine calcaneal SI using QUS (Achilles Lunar Insight) in a large sample of more than 10,000 children in a cross-sectional and longitudinal perspective. This thesis primarily focuses on the SI measurements in a cross-sectional perspective considering children 2-10 years of age with their first QUS measurement at T0 (N = 7,539) or T1 (N = 3,842) and thus provides first insights into the relevance of calcaneal SI in childhood.

BUA and SOS measurements were not available for T0 and were thus not considered in the context of this work. However, both parameters were assessed during T1 of the IDEFICS study and in the I.Family study.

In the IDEFICS study, QUS measurements were performed once each on the right and left foot. The mean SI from the left and right measurement was used for statistical analyses as a proxy for the bone status of the lower limbs and hip. Another reason for using the mean SI was its better observed precision compared to the precision of the SI of either the right or left foot alone, as described in *Chapter 5.2.3.1*.

Based on the Operation Manual from the manufacturers, standardized measurement procedures were developed for all countries within a General Survey Manual.[89] In brief:

"[…] For standardized measurement, the child was asked to sit barefoot on a stable chair directly in front of the device. The leg was positioned so that the foot and calf were aligned with the foot positioner. The foot was positioned using an adapter for children's feet to put the

calcaneus in focus. The study nurse made sure that the foot was flat and positioned firmly against the bottom of the footplate and that the child was not moving during the measurement. When changing the measured foot, the position on the chair had to be adjusted. Quality control was performed weekly, using the implemented quality assurance test of Achilles Insight." (Herrmann et al., 2014, p.77)[30,89]

Furthermore, 70% isopropyl alcohol was used as the contact agent. No adjustment for the region of interest (ROI) was made. Exclusion criteria for QUS measurements were infections (e.g. mycosis) or injuries on the foot. Study nurses of each participating country were trained in terms of using the Achilles devices according to the standardized procedures in the General Survey Manual.

5.2 Reliability of the Measurements of Bone Stiffness

5.2.1 Background

According to literature, the rate of change in bone density varies between 0.5% and 2% per year.[66] Imaging technologies must therefore be able to reliably detect such minor changes in bone indices to be effective in clinical settings monitoring the progression of bone-related diseases or medical treatments.[107] Although small variations in the measurements are unavoidable, a certain precision, i.e. "the ability to reproduce the quantitative measurement […] under identical circumstances" of clinical tests needs to be warranted (Hawkinson et al., 2007, p. 10).[66]

In addition to the reliability of the SI measurements of one device, the comparability of the SI measurements between various QUS devices is of relevance. Over the years, different devices may be used to monitor bone diseases or to assess bone health in clinical practice. Within cohort studies, the application of more than one QUS device is unavoidable as this involves large population samples.[95] Therefore, there is a need to ensure that the different QUS devices used are comparable. To date, this issue has hardly been investigated.

The main problems concerning the reliability of SI measurements obtained using the same or using different devices may be related to potential error measurements such as poor positioning of the individual or to the fact that the measurement tools and procedures are not used according to pre-defined standards.[95] The extent to which SI values derived with these error measurements vary from SI values measured according to standardized procedures has not yet been investigated.

Currently there are no recommendations on which SI value of which foot should be used as an indicator for bone status. This could explain why SI values in previous studies on bone health were not reported uniformly. Some of them reported only for the left or right foot, or some of them reported the mean SI value of both feet.[95,96,100-103,108] Potential differences in the SI values between the left and right foot could thus have remained undetected, thereby hindering the comparability of results between studies. Explorative analyses of the IDEFICS population showed an absolute SI difference between the right and left foot ranging from 0-112 units (see Table 3). These findings would not have been observed had only one foot been measured.

In the context of this work, the reliability of SI measurements in children and adults was studied as described in the following four objectives.

1) To assess the reproducibility of repeated SI measurements in a QUS device.
2) To analyse the comparability of the SI measurements between the QUS devices used in the IDEFICS study.
3) To assess the deviation of SI values derived with error measurements compared to SI values measured according to standardized procedures (= standard measurement).
4) To determine the magnitude of the variation of SI values between the left and right foot.

To examine the reliability of imaging techniques, the Conference of Radiation Control Program Directors (CRCPD) Task Force on Bone Densitometry recommends that at least two measurements of one skeletal site should be done on 30 individuals, preferably on the same day, with repositioning before the repeated measurement.[66] This was done in the following studies that were conducted for investigating the reliability and comparability of SI measurements in the IDEFICS study.

5.2.2 Methods

5.2.2.1 Study Samples

The reproducibility of repeated SI measurements in children was analysed in a sub-sample from the IDEFICS study. Two further studies in adults were conducted to examine the comparability of SI measurements between the QUS devices as well as to analyse potential variations of SI values due to error measurements, or respectively between the left and right foot.

The sub-sample from the IDEFICS study consisted of 60 children 5.6-9.3 years of age from the baseline survey of the IDEFICS study (sample 1). Repeated measurements were performed on either the left (N = 30) or the right foot (N = 30). These measurements were used to examine the precision, i.e. reproducibility of the repeated SI measurements in children.

To examine the comparability of SI measurements between different QUS devices from the IDEFICS study, a further study was conducted in 2012 in 91 colleagues from the Leibniz Institute for Prevention Research and Epidemiology, BIPS including their family or friends (sample 2). The participants were aged 20-71 years. For this study, five of the eight QUS devices used in the IDEFICS study (i.e. from Germany, Sweden, Italy, Estonia and Cyprus) were available. In each participant, three measurements were performed on both, on the left as well as on the right foot using each of the available QUS devices. This resulted in a total of 30 measurements per participant. The procedure in each individual lasted about 90 minutes.

A further study in adults was conducted in 2011 to investigate the difference of SI values between potential error measurements and standard measurements. This convenience study sample consisted of 12 voluntary colleagues from BIPS aged 22-49 years (sample 3). The variations between error and standard measurements were examined using two available Achilles devices from the IDEFICS study. Following four error measurements were considered: a) no manual adjustment of the region of interest, b) less alcohol on the membranes, i.e. one sprayer per membrane, c) wrong foot position, i.e. holding the foot on the right membrane and d) the use of a wrong inlay, i.e. for children. In each participant, three standard measurements and one measurement of each error condition were performed on both, on the left as well as on the right foot using each device. Thus, each participant had a total of 28 measurements, which took about 90 minutes.

The exclusion criteria applied for the IDEFICS study were also applicable for participation in the three reliability studies. A further exclusion criterion for samples 2 and 3 was the pregnancy in women.

5.2.2.2 Definition of Variables

To investigate the reproducibility of the SI standard measurements in a single device, the variable ‘repeated measurements’ was categorised in terms of the number of the repeated standard measurements per participant, per device and per foot in children (two repeated standard measurements) and adults (three repeated standard measurements).

In order to compare the SI measurements between the different QUS devices, the variable 'QUS devices' was categorised as follows: i) QUS 1 Germany, ii) QUS 2 Sweden, iii) QUS 3 Italy, vi) QUS 4 Estonia and v) QUS 5 Cyprus.

To examine differences between error and standard measurements the variable 'error measurements' was categorised as follows: i) standard measurement, ii) no adjustment, iii) less alcohol, iv) wrong foot position and v) wrong inlay.

Differences of SI measurements between the left and right foot were analysed using the dichotomous variable 'measured foot' (left versus right).

5.2.2.3 Reproducibility and Precision Error

For examining the reproducibility of an SI measurement, the precision error was estimated based on the coefficient of variation (CV) and the root-mean-square CV (CV_{RMS}) in children and adults (samples 1 and 2). Both parameters were expressed as percent (%). The ISCD defines the CV as "a unitless number which expresses the variability of a normal distribution" (ISCD, 2013, Glossary of Terms).[65] The CV is the ratio of the SD divided by the mean of the repeated measurements in an individual and is calculated as:

$$CV_i = \frac{SD_i}{\bar{x}_i}$$

In this equation $\bar{x}_i$ is the mean value of the repeated measurements of an individual per foot and per device. SD_i is the SD of the repeated measurements. In this work, the average CV is presented, which is based on all individual CVs of each sample.

Although most studies report the CV, the CRCPD and ISCD recommend reporting the CV by calculating CV_{RMS}:[66,80]

$$CV_{RMS} = \sqrt{\frac{\sum_{i=1}^{m}(CV_i^2)}{n}}$$

In this equation n is the number of individuals included in the samples. The calculation of CV_{RMS} was conducted as follows (description adapted from Hawkinson et al., 2007, p. 12):[66]

i. The CV of each individual is squared;
ii. The individual CV values are summed up;
iii. The sum is divided by the number of individuals (n);
iv. The square root is taken.

5.2.2.4 Statistical Analysis

Multilevel regression analysis (using the procedure GLIMMIX) was used to examine the differences of the SI values between 1) the repeated standard measurements per participant, per foot, per device, 2) the different QUS devices, 3) the error versus standard measurements as well as 4) the left and right foot. In sample 1, the model (M1) included fixed effects for the variable 'repeated measurements' (reference: measurement 1). The model for sample 2 (M2) included fixed effects for the variables 'repeated measurements' (reference: measurement 1), 'QUS devices' (reference: QUS 1 Germany) and 'measured foot' (reference: right). In addition to M2, the fixed effects for the variable 'error measurements' (reference: standard measurement) were added in the analysis of sample 3 (M3). All models included a subject-specific random intercept, and SI (continuous) was the dependent variable.

The level of significance for all analyses was set at $\alpha = 0.05$. The analyses were performed using SAS 9.3 (SAS Institute Inc., Cary, NC, USA).

5.2.3 Results

Table 3 presents the mean value, SD and value range of the age as well as of the SI measurements from the left and right foot, the mean SI (of the left and right foot) and the absolute difference between the left and right SI measurement for the samples from the reliability study. In addition, these characteristics are shown for all children from the IDEFICS study with their first QUS measurement from T0 or T1.[30]

As it was expected, SI values in adults were higher compared to children. In all samples of the reliability study, slightly higher SI values for the right foot compared to the left foot were observed, considering the fact that in children in sample 1 the left and right foot was not

Table 3 Characteristics of the samples of the reliability analyses

	IDEFICS children (N = 11,381)*		**Sample 1 (children) (N = 30 per foot)**		**Sample 2 (adults) (N = 91)**		**Sample 3 (adults) (N = 12)**	
	Mean±SD	**range**	**Mean±SD**	**range**	**mean±SD**	**range**	**Mean±SD**	**range**
Age (years)	6.7±2.0	2-10	7.2±0.9	6-9	37.8±12.9	20-71	30.9±7.8	22-49
SI left	81.2±16.1	40-166	78.0±15.7	56-116	103.3±18.3	63-165	101.6±14.5	69-139
SI right	81.2±16.3	40-163	78.8±16.8	47-118	103.8±18.4	69-164	101.8±13.0	76-128
SI mean	81.2±14.0	46-153	-	-	103.5±17.8	66-158	101.7±12.7	73-128
SIdiff	6.4±6.4	0-112	-	-	6.4±6.4	0-55	8.1±6.2	0–29

* Characteristics of children from the IDEFICS study having their first SI measurement in either T0 or T1.[30]

measured in one individual. Thus, no intra-individual comparison of the left and right SI measurement should be made with this sample. However, SI values for the right foot compared to the left foot were equal for the children from the IDEFICS cohort. Nevertheless, in the latter the absolute difference between the left and right foot in the individuals ranged from 0-112 units. In contrast, the absolute difference between the right and left foot in adults varied from 0-55 units (see Table 3).

Table 4 presents the CV_{RMS} and CV for the SI measurements for all samples. Table 5 shows the estimates (β), p-values and 95% confidence intervals for the differences of the SI measurements for 1) the repeated measurements per foot per device, 2) the different QUS devices, 3) the standard versus error measurements and 4) the left versus right foot.

5.2.3.1 Reproducibility of SI Measurements

In children, the CV_{RMS} for the SI measurements on the left foot was 7.2% and on the right foot 9.2%. In contrast, in adults (sample 2), the CV_{RMS} for the SI on the left foot was 2.2% and on the right foot 2.0%. The CV_{RMS} for the mean SI value of both feet in adults was 1.5%.

The averaged CV per sample showed lower values compared to the CV_{RMS} (see Table 4). The range of the individual CVs was 0.3% - 21.2% in children and 0.02% - 11.9% in adults.

Multilevel regression analysis showed no significant difference of repeated SI measurements compared to the first measurement in children. However, a higher estimate was observed in children ($\beta_{measure2} = -1.44$, $p = 0.23$) compared to adults from sample 2 ($\beta_{measure2} = 0.13$, $p = 0.63$; $\beta_{measure3} = 0.27$, $p = 0.32$; see Table 5).

Table 4 Percent root-mean-square coefficient of variation (CV_{RMS}) and averaged percent CV of the SI measurements on the left and right foot using Achilles Insight

	CV_{RMS} (%)			CV (%)		
	Left foot	**Right foot**	**Mean[1]**	**Left foot**	**Right foot**	**Mean[1]**
Children (sample 1)	7.2%	9.2%	-	4.9%	8.0%	-
Adults (sample 2)	2.2%	2.0%	1.5%	1.4%	1.5%	1.1%

[1] CV / CV_{RMS} (%) of the mean SI from the SI values of the left and right foot

Table 5 Multilevel regression analysis estimating the difference in SI (β) between the 1) repeated standard measurements, 2) different QUS devices, 3) error versus standard measurements and 4) left versus right foot

Dependent variable: SI	Sample 1 (N = 60)			Sample 2 (N = 91)			Sample 3 (N = 12)		
	β	*p*-value	95%-CI	β	*p*-value	95%-CI	β	*p*-value	95%-CI
1) Repeated measurements: *Reference: Measurement 1*									
Measurement 2	-1.44	0.23	-3.81; 0.93	0.13	0.63	-0.41; 0.68	0.47	0.75	-2.41; 3.36
Measurement 3	-	-	-	0.27	0.32	-0.27; 0.82	0.34	0.81	-2.54; 3.23
2) QUS devices: *Reference: QUS 1 Germany*									
QUS 2 Sweden	-	-	-	2.74	<.001	2.06; 3.42	2.80	<.001	1.26; 4.35
QUS 3 Italy	-	-	-	-1.63	<.001	-2.31; -0.95	-	-	-
QUS 4 Estonia	-	-	-	-2.47	<.001	-3.25; -1.68	-	-	-
QUS 5 Cyprus	-	-	-	2.82	<.001	2.14; 3.50	-	-	-
3) Error measurements: *Reference: Standard measurement*									
No adjustment	-	-	-	-	-	-	2.24	0.06	-0.11; 4.59
Less alcohol	-	-	-	-	-	-	-0.67	0.57	-3.02; 1.68
Wrong foot position	-	-	-	-	-	-	-3.40	0.005	-5.74; -1.05
Wrong Inlay	-	-	-	-	-	-	-4.72	<.001	-7.07; -2.37
4) Measured foot: *Reference: Right*									
Left	-	-	-	-0.45	0.05	-0.89; -7.02^{-6}	-0.26	0.74	-1.79; 1.28

5.2.3.2 Comparison of Different Achilles Insight Devices

SI measurements between the devices partly differed significantly from each other. The differences in SI values varied between -2.5 and 2.8 units, taking the device from Germany as reference. The results indicate the smallest difference in the average SI, i.e. a difference of 0.08 units between the devices from Sweden and Cyprus. In contrast, the largest difference of 5.3 units in the average SI was observed between the devices from Estonia and Cyprus (see Table 5).

5.2.3.3 Error and Standard Measurements

Using the wrong inlay, i.e. the inlay for child's feet in adults, resulted in a significant difference in SI of -4.7 units *($p < .001$)* compared to the standard measurement. In addition, the wrong positioning of the foot in the device led to a significant discrepancy of the SI measurement ($\beta = -3.4$, $p = 0.005$). In contrast, the SI was not significantly different from the standard measurement when the region of interest was not adjusted or less 70% isopropyl alcohol was used.

5.2.3.4 Variations of SI between the Left and Right Foot

Since SI was not measured on the left and right foot in children from sample 1, the difference of SI could only be examined in adults. Results of multilevel regression analysis in sample 2 showed in average a significant but weak lower SI of 0.45 units in the left foot compared to the right foot ($p = 0.05$, see Table 5). No significant SI difference was observed in sample 3.

5.2.4 Discussion

5.2.4.1 Reproducibility of SI Measurements

Previous studies that examined the precision of Achilles devices in children observed smaller CV_{RMS} ranging from 3.5% - 3.8% compared to those found in the present study. However, these studies had smaller sample sizes and included children and adolescents 5-15 years of age.[103,109]

The larger precision error observed in children compared to adults might be explained by small movements of the children during the examination or by a different or incorrect positioning during the repeated measurements. In the current reliability study, the incorrect position of the foot was observed to significantly underestimate the SI measurement by approximately 3.4 units. Nevertheless, despite the relatively high precision error in children compared to adults, the differences between repeated SI measurements rather small in children as well as in adults.

Comparing the CV_{RMS} from our study with previous studies is challenging as previous studies mainly reported the percent CV for SI measurements. In younger individuals, they observed a CV varying from 1.8% - 2.5%. However, they mostly examined study populations including children and adolescents.[96,101-104] In adults, the reported CV varied from 1.3% - 1.9%, which is in line with the CV observed in this thesis.[95,97,110,111]

A comparison of the precision between QUS and DXA measurements in children suggests a better precision of DXA measurements (CV = 0.8% - 1.3%).[112] In adults, the CV of DXA measurements of the hip ranged from 0.8% - 3.3%, which did not differ essentially from the CV of calcaneal SI measurements of the adult sample 2 in this work.[113]

5.2.4.2 Comparison of Different Achilles Insight Devices

The small differences of SI measurements observed between the devices from Sweden and Cyprus, or respectively between the devices from Italy and Estonia were in line with the

reported differences in a study from Economos et al. who investigated the precision of the measurements of two Achilles+ devices in 13 young adults (27.6±4.6 years). The researchers reported a difference of 0.5 units ($p = 0.01$) between the SI measurements of the two devices.[95] Nevertheless, in our sample 2 much larger differences between SI measurements from different devices ranging from 2-5 units were observed suggesting that longitudinal analyses using individual-based data from different QUS devices should be interpreted with caution. For instance, the results presented in *Article 2* indicate a 1.2-1.6 unit higher SI in children with each additional 10 minutes in VPA *(see App. B)*. Although these results are cross-sectional, such a significant increase in SI, for instance after intervention would not be detected, when monitoring bone health in one individual with different QUS devices that vary in their SI measurements by 2 units or more.

The differences between the measured SI values of the IDEFICS devices may partly be a consequence of the process of their transportation between survey centres, respectively their shipment from abroad to Germany. Furthermore, the fact that the last reliability study of the QUS devices had been conducted almost five years after the IDEFICS baseline survey should also be taken into consideration. Long-term precision of the QUS devices after one year were only reported by Economos et al. who did not observe any significant difference in SI measurements of Achilles+.[95] However, the precision after a period of at least two years or more and thus the reliability over time of Achilles or other QUS devices remain unknown.

5.2.4.3 Error and Standard Measurements

The results with respect to the error measurements suggest an underestimation of the SI values when applying the wrong inlay or incorrect positioning the foot. On the other hand, the results indicate that using the correct inlay and positioning the foot correctly, the region of interest for measuring the calcaneus is normally in a good position. This supports the standardized measurement procedure followed in the IDEFICS study, where the region of interest was not adjusted. Adjusting the region of interest in large observational studies may be a disadvantage in terms of the time and the feasibility of standardization when no radiologists or similar experts are available. However, the magnitude of discrepancy in SI measurements when adjusting versus not adjusting the region of interest of the calcaneus in children needs to be further investigated.

5.2.4.4 Variations of SI between the Left and Right Foot

The descriptive and analytic results in this work on reliability showed slightly lower SI values in the left compared to the right foot. From a biological perspective, that may be explained by the fact that the right foot is usually being the dominant site in most individuals. However, no evidence of this hypothesis has been found, i.e. this may also require further investigation.

Nevertheless, SI differences of up to 100 units between the left and right foot in the same child appear questionable. Such differences may be partly explained by error measurements such as the wrong positioning of the foot, as observed in the current analysis. The lack of studies examining SI of the left and right foot makes it difficult to draw conclusions on the plausibility of the observed discrepancy. Hence, in a first step, the distribution of the absolute SI differences between the left and right foot was examined calculating percentile values based on all IDEFICS children with SI measurements (N = 11,381; see Table 6). To omit potential implausible values or erroneous measurements and to retain at the same time sufficient power for the derivation of reference values and statistical analyses, 3% of children having the highest absolute SI differences, i.e. a difference in SI measurements of more than 45 units between the left and right foot were excluded.

Table 6 Percentiles and SI values of the absolute difference of the SI measurement between the left and right foot (based on 11,381 children with QUS measurements)

Percentiles	**50**	**75**	**90**	**95**	**97**	**99**
Absolute SI difference	6	13	26	38	45	59

The large observed difference between the left and right foot in the IDEFICS children suggest to use the mean SI value of the left and right SI measurement as an indicator for bone status in the lower limbs for statistical analysis. A further reason for using the mean SI value lies in the better precision of the mean SI compared to the precision of either the left or right SI measurement, alone.

In conclusion, changes in SI values in a child during the monitoring of the progression of bone-related diseases or medical treatments may be detected when using the same QUS device and positioning the foot correctly. Furthermore, QUS is also an alternative method to DXA in epidemiological studies for investigating bone health and its determinants in children in order to reach a sufficient sample size. However, the comparability of the SI measurements of different used devices must be ensured. In contrast, the higher precision error of SI measurements in children as well as the underestimation due to error measurements and the

potential variations of SI values between the devices support the ISCD recommendation, that QUS should not be used for diagnosing osteoporosis.[80]

This thesis cannot close the gap of research regarding the reproducibility of Achilles measurements in children. Further studies need to be carried out with respect to the precision of the mean SI from the measurements from the left and right foot, and the influence of potential error measurements on SI values.

6 Strengths and Limitations

A major strength of this thesis is the large sample of apparently healthy European children with standardized data for SI measurements, biochemical markers, anthropometric measurements and different lifestyle behaviours. Furthermore, the detailed examination of the health and medical history of the children allowed the exclusion of children who suffered from bone-related diseases or who received medical treatment known to affect bone metabolism. This comprehensive database was especially important for the derivation of the age-, sex- and height-specific reference values on SI and CTX and the analysis of the case-control study in this work.

Furthermore, the large examination programme of the IDEFICS study comprises subjectively and objectively assessed data. From a methodological perspective, the comparison of different assessment methods is important for analysing their informative value with regard to certain health outcomes. This is essential when aiming to derive dose-response-relationships.

However, the lack of longitudinal analyses for this thesis limits the interpretation of the findings with respect to causality, adequate dose-response relationships between PA and SI and of the sustainable osteogenic effect of PA.

The fact, that no data were available on fracture history in the IDEFICS children, is a further weakness of this work. According to the ISCD, these information help to indicate the fracture risk in children. The data are of interest for the investigation of the informative value of QUS measurements in children who already have an increased risk of fractures.

In addition, only data on CTX as a marker of bone resorption were available. The combination of CTX with at least one formation marker, as recommended by the IOF and IFCC, would be more precise to indicate bone turnover in children.[42]

The incomplete data on BUA and SOS, due to different registration settings of the QUS devices at T0 are a further limitation. Although a better precision of SI compared to BUA and SOS has been reported in some studies,[93] others observed significant association of determinants or fracture risk with BUA and SOS rather than with SI *(Article 7, see App. G)*.[78,103,114,115] Thus, the informative value of BUA and SOS on bone health is still not clear and should be further investigated.

Finally, the data on the reliability of the SI measurements in children have some restrictions. In further reliability studies, SI measurements should be performed on the left and right foot

of each child to enable the examination of the precision of the mean SI of both feet. Furthermore, data on error measurements in children may help to avoid inadequate handling during QUS procedures, and thus help to increase precision.

7 Conclusion

This thesis adds age-, sex- and height-specific reference data on calcaneal SI and serum CTX for children aged 2-10 years. These reference data give first insights into the age-specific distribution of SI and CTX values. They furthermore form the basis for comparing bone health of children in further epidemiological studies and clinical settings with a healthy European reference population.

The observed associations between PA and SI in this thesis are in line with the general understanding of bone physiology and with results from previous studies that assessed bone health using x-ray imaging technologies. For instance, the findings of this work suggest a strong association of high-impact PA and a weak or negative association of light PA or sedentary behaviour with SI. The osteogenic effect of calcium and vitamin D levels within a normal range however appeared to be negligible. The comparable findings from this thesis with those of previous studies support the use of QUS in future epidemiological studies and clinical settings to investigate the effects of potential risk and protective factors on bone health as well as for monitoring bone status during interventions in children.

However, the reliability and comparability of the SI measurements assessed with the same or with different devices must be taken into account, especially when measurements were taken in children. The question concerning the reliability of SI measurements in children was not fully answered in this work and thus needs to be further investigated.

Given the reported limitations of this thesis, randomized controlled trials or clinical studies based on children with a significant fracture history or bone-related diseases would be desirable. These trials could help to investigate the usefulness of SI measurements for prediction of fracture risk and diagnosing paediatric osteoporosis. Furthermore, longitudinal studies are needed to fill the gap of knowledge on the sustainable effect of high-impact PA on bone health and to fully understand the factors explaining variation of SI in children. Longitudinal QUS data from the follow-up surveys of the IDEFICS and I.Family study enable the investigation of SI over a time span of up to six years, covering the transition phase from childhood into early adolescence. Analyses of that data may give first evidence on whether SI values are suitable in terms of monitoring skeletal development and detecting sustainable osteogenic effects in relation to changes in PA behaviour.

Furthermore, it would be desirable to extend the reference data on SI and CTX into early adulthood to allow monitoring of skeletal development until peak bone mass is reached. SI

measurements and serum CTX from the I.Family study may be a starting point to amend the reference data on SI and CTX into adolescence.

From a public health perspective, monitoring bone health in young individuals, especially in those with a history of fractures, can help to identify deviations from a normal skeletal development. This would allow the implementation of early interventions in order to increase peak bone mass.

Finally, the application of QUS in epidemiological studies not only helps to further investigate methodological questions of QUS measurements. It may also help sensitise people to pay more attention to their bone health before the onset of osteoporosis or before osteoporotic fractures occur.

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Articles in Thesis

The cumulative dissertation is based on following six original articles and a review which are printed in kursiv where they are referred to in the text and are to be found in the Appendix.

Article 1:

Herrmann D, Hebestreit A, Ahrens W. Impact of physical activity and exercise on bone health in the life course: A review. *Bundesgesundheitsblatt Gesundheitsforschung Gesundheitsschutz.* 2012;55(1):35-54. DOI: 10.1007/s00103-011-1393-z.

Article 2:

Herrmann D, Buck C, Sioen I, Kouride Y, Mårild S, Moreno LA et al. Impact of sedentary behaviour and muscular strength on bone stiffness in 2-10-year-old children. Cross-sectional results from the IDEFICS study. *International Journal of Behavioural Nutrition and Physical Activity* (submitted).

Article 3:

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 pre-adolescent children. A case-control study. Bone. 2015;78:142-149. DOI: 10.1016/j.bone.2015.04.043.

Article 4:

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. DOI: 10.1038/ijo2014.138.

Article 5:

Gianfagna F, Cugino D, Ahrens W, Bailey ME, Bammann K, **Herrmann D** et al. Understanding the links among neuromedin U gene, beta2-adrenoceptor gene and bone health: an observational study in European children. *PLoS One.* 2013;8(8):e70632. DOI: 10.1371/journal.pone.0070632.

Article 6:

Sioen I, Mouratidou T, **Herrmann D**, De Henauw S, Kaufman JM, Molnár 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):276-285. DOI: 10.1007/s00223-012-9640-3.

Article 7:

Van den Bussche K, Michels N, Gracia-Marco L, **Herrmann D**, Eiben G, De Henauw S, Sioen I. Influence of birth weight on calcaneal bone stiffness in Belgian preadolescent children. *Calcif Tissue Int.* 2012;91(4):267-275. DOI: 10.1007/s00223-012-9636-z.

Appendix

Appendix A

Impact of Physical Activity and Exercise on Bone Health in the Life Course: A Review

Herrmann D, Hebestreit A, Ahrens W

Bundesgesundheitsblatt Gesundheitsforschung Gesundheitsschutz. 2012;55(1):35-54.
DOI:0.1007/s00103-011-1393-z

Einfluss von körperlicher Aktivität und Sport auf die Knochengesundheit im Lebenslauf - Ein Überblick

Kurzüberschrift

Knochengesundheit und körperliche Aktivität

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Zusammenfassung

Körperliche Aktivität und Sport sind nicht nur für die metabolische und kardiovaskuläre Gesundheit, sondern auch für die Knochengesundheit von großer Bedeutung. Der Überblick fasst Ergebnisse aus Beobachtungs- und Interventionsstudien zusammen, die den Zusammenhang zwischen körperlicher/sportlicher Aktivität und der Knochengesundheit im Lebenslauf untersuchen. Bereits im Kindes- und Jugendalter führen körperliche Aktivität und Sport zu erhöhtem Knochenzuwachs. Im Erwachsenenalter kann altersbedingter Knochenschwund durch kontinuierliche und über mehrere Monate durchgeführte Sportprogramme vermindert werden. Dabei zeigen insbesondere weight-bearing activities einen bedeutenden osteogenen Effekt. Im Kindes- und Jugendalter ist ein höherer Knochenzuwachs bis zu fünf Jahren nach Beendigung des Sportprogramms zu beobachten. Im Erwachsenenalter nimmt die Knochenfestigkeit nach Beendigung des Sports rapider ab, als bei Nicht-Sportlern. Kontinuierlich durchgeführte körperliche und sportliche Aktivität sowie die Implementierung von Sportprogrammen in Schulen und bevölkerungsbasierten Interventionsprogrammen sind präventive Maßnahmen, um Osteoporose und Osteoporose bedingten Frakturen vorzubeugen. Aufgrund fehlender prospektiver Langzeituntersuchungen ist die vermutete langfristig anhaltende Schutzwirkung von hoher körperlicher Aktivität und Sport im Kindes- und Jugendalter auf den altersbedingten Knochenschwund im Erwachsenenalter noch nicht belegt.

Schlüsselwörter: Knochengesundheit, Osteoporose, körperliche Aktivität, Sport, Kompetenznetz Adipositas

Impact of physical activity and exercise on bone health in the life course. A review

Abstract

Physical activity and exercise are important determinants for metabolic and cardiovascular health. They play also an important role for bone health in childhood, adolescence and adulthood. This review summarizes results from observational and intervention studies, which evaluated the association between physical activity/exercise and bone health in different life course stages. In childhood and adolescence, physical activity and exercise induced an improved bone accrual. In adulthood, mainly in postmenopausal women, long-term exercise programs reduced age-related bone loss. Especially weight bearing activities had an important osteogenic effect. Children and adolescent show a higher bone accrual until five years after cessation of an exercise program compared to their peers, who do not participate in an exercise program. Adults who quit their exercise have a higher decrease in bone stiffness compared to non-exercisers. This effect was particularly seen in postmenopausal women. Continuous physical activity and exercise over the life course and the implementation of exercise programs in schools and community based intervention programs can help to prevent or even reduce osteoporosis and osteoporosis related fractures. Due to the lack of prospective longitudinal studies, the supposed long-term sustainable protective effect of physical activity and exercise in childhood and adolescent on bone health in later adulthood is not well established.

Keywords: bone health, osteoporosis, physical activity, exercise, Competence Network Obesity

Einleitung

Zahlreiche aktuelle Beobachtungs- und Interventionsstudien beschreiben die Bedeutung von körperlicher Aktivität und Sport für die Knochengesundheit. Bereits im Kindes- und Jugendalter erhöht sich der Knochenzuwachs infolge körperlicher und sportlicher Aktivität. Im Erwachsenenalter, vor allem bei Frauen nach der Menopause können altersbedingte Knochenabbauprozesse durch kontinuierlich durchgeführte Sportprogramme verlangsamt werden (1-17). Die Wirkungsweise körperlicher Aktivität und Sport auf die Knochenanbau- und Knochenumstrukturierungsprozesse ist Gegenstand zahlreicher Untersuchungen (18-23).

Aktuelle Übersichtsarbeiten fassen überwiegend Interventionsstudien, vor allem randomisierte kontrollierte Studien (RCT) zusammen, welche die Wirkung bestimmter Sportarten und -programme auf den Knochenzuwachs in verschiedenen Lebensphasen untersuchen. Nur selten wurde dagegen der Einfluss alltäglicher körperlicher Aktivität untersucht. Zudem gibt es nur wenige Arbeiten, die den Einfluss von körperlicher Aktivität oder Sport im Kindes-, Jugend- *und* Erwachsenenalter, das heißt im gesamten Lebenslauf dar- und gegenüberstellen. Obwohl die verschiedenen Lebensphasen getrennt voneinander untersucht werden, wird häufig geschlussfolgert: körperliche Aktivität und Sport im Kindes- und Jugendalter erhöhen den Knochenzuwachs, wodurch das Risiko für Erkrankungen des Bewegungsapparates, wie Osteopenie, Osteoporose und osteoporosebedingte Frakturen im Erwachsenenalter reduziert wird (18;19;24;25).

Diese narrative Übersichtsarbeit fasst den gegenwärtigen Wissensstand zum Einfluss und zur Wirkungsweise körperlicher *und* sportlicher Aktivität auf die Knochengesundheit im Kindes-, Jugend- *und* Erwachsenenalter, das heißt im gesamten Lebenslauf zusammen, wobei Überblicksartikel, Interventions- und Beobachtungsstudien ab dem Jahr 2000 eingeschlossen werden. Neben Besonderheiten und Limitationen der Studiendesigns werden mögliche sensitive Zeitfenster für nachhaltige Interventionsstrategien im Lebenslauf diskutiert.

Hintergrund

Die kritischste Eigenschaft, die einen Knochen als gesund oder krank beschreibt ist die Knochenfestigkeit (21). Knochenarchitektur und -struktur sind wichtige Charakteristika der Knochenfestigkeit, die an unterschiedlichen Knochen und Knochenregionen mithilfe verschiedener Indikatoren beschrieben werden (21;23). Aufgrund der hohen alters- und bewegungsabhängigen Abnutzungserscheinungen, werden häufig Indikatoren an Knochenregionen der Extremitäten, Hüfte sowie der Lendenwirbelsäule als Endpunkte zur Erforschung der Knochenfestigkeit verwendet (26;27). Die am häufigsten untersuchten

Knochenregionen und Indikatoren werden in Tabelle 1 dargestellt und sind in diesem Übersichtsartikel mit dem Begriff der „Knochenfestigkeit“ (KF) zusammengefasst und definiert.

Die größte mechanische Einwirkung auf den Knochen hat die Muskelkontraktion, ausgelöst durch körperliche Aktivität (18;20). Zwischen Muskelkraft und KF besteht eine stärkere Assoziation, als zwischen Alter oder Körpergewicht und KF (21;22). Es ist erwiesen, dass langandauernde körperliche Inaktivität (Bettruhe) nicht nur zu Muskel- sondern auch zu Knochenschwund führt. Infolge der reduzierten Knochenmasse und -dichte und einer zerstörten Mikroarchitektur ist das Risiko für Osteoporose und Osteopenie, die Vorstufe von Osteoporose, erhöht (19;28). Osteoporose ist heutzutage ein bedeutendes gesellschaftliches Gesundheitsproblem von dem viele Millionen Menschen, vor allem postmenopausale Frauen, weltweit betroffen sind (27). Die WHO definiert Osteoporose bei postmenopausalen Frauen und Männern über 50 Jahren ab einer um 2.5 Standardabweichungen reduzierten Knochendichte (*Bone Mineral Density*, BMD) im Vergleich zu jungen gesunden Frauen (20-29 Jahre). Die klinische Relevanz Osteoporose zu erfassen liegt im erhöhten Frakturrisiko an Oberschenkelhals, Hüfte und Lendenwirbelsäule. Zudem führen sowohl Osteoporose als auch osteoporosebedingte Frakturen zu einer erhöhten Morbidität und Mortalität (26;27).

Zahlreiche Beobachtungs- und Interventionsstudien haben den Einfluss von körperlicher Aktivität und Sport auf die Knochengesundheit untersucht (Tabellen 2-5). Dabei zeigen Querschnittsstudien lediglich Zusammenhänge zwischen körperlicher Aktivität und KF. Prospektive Beobachtungsstudien und experimentelle Untersuchungen hingegen können mögliche Veränderungen und Wirkungen infolge körperlicher und sportlicher Aktivität auf den Bewegungsapparat darstellen. Um Studienergebnisse miteinander vergleichen zu können müssen die Begriffe „körperliche Aktivität“ und „Sport“ unterschieden werden. Unter körperliche Aktivität (KA) ist jede körperliche Bewegung zu verstehen, bei der die Skelettmuskulatur durch eine Muskelkontraktion beansprucht wird und die einen Energieverbrauch zur Folge hat. Sport ist eine geplante, strukturierte und sich wiederholende körperliche Aktivität, die eine Verbesserung der körperlichen Fitness anstrebt (29). Der Effekt der Muskelkontraktion auf die KF ist von Art, Dauer und Intensität der KA abhängig (30). Vor allem kurzzeitig belastende und gewichtstragende Aktivitäten (*Weight-Bearing Activities*, WBA) mit einer hohen Bodenreaktionskraft (*Ground Reaction Force*, GRF) lösen starke Muskelkontraktionen in den unteren und oberen Extremitäten, in der Hüfte sowie in der Lendenwirbelsäule aus und haben auf diese Knochenpartien eine osteogene

(knochenaufbauende) Wirkung (7;9;25;31-37). Bekannte WBA mit einer hohen GRF und einem nachgewiesenen Einfluss auf die KF sind Fußball, Kurz- und Mittelstreckenläufe (GRF: 2-3-fache des Körpergewichts), Basketball (GRF: 3.9-4.6-fache des Körpergewichts) oder Volleyball (GRF: 3-6-fache des Körpergewichts) (38). Hingegen wurden für geringe gewichtstragende Aktivitäten mit einer niedrigen GRF (Schwimmen, Radfahren) keine osteogenen Effekte ermittelt (5;13;25;31;37). Während mechanische Belastungen die Variabilität der KF bis zu 40% determinieren, bestimmen nicht-mechanische Faktoren, wie Hormone (Wachstumshormone, Androgene) und Nährstoffe (Kalzium, Vitamin D) die Anbau- und Umstrukturierungsprozesse im Knochen zu einem geringeren Prozentsatz. So wurde festgestellt, dass bei gesunden Menschen ohne Hormonmangel, ohne eine klinisch relevante Rachitis oder ohne Erkrankungen des muskuloskelettalen Systems die Variabilität der KF durch nicht-mechanische Faktoren bis zu 10% determiniert werden kann (20;21).

Einfluss KA auf die Knochengesundheit im Kindes- und Jugendalter

Das Kindes- und Jugendalter wird als eine der sensibelsten Phasen für Aufbau- und Umstrukturierungsprozesse des Knochens beschrieben (18;34). Eine optimale Knochenentwicklung wird häufig mithilfe der maximalen Knochenmasse (*peak bone mass*, PBM) geschätzt, die zwischen dem 20.-30. Lebensjahr erreicht wird. In der Knochenforschung wird infolge einer hohen erreichten PBM eine verbesserte KF sowie ein präventiver Effekt für das spätere Fraktur- oder Osteoporoserisiko im Erwachsenenalter zugeschrieben (14;19;39). Obwohl die PBM zu einem hohen Anteil genetisch festgelegt ist, weisen Beobachtungs- und Interventionsstudien darauf hin, dass KA und Sport die PBM in jungen Jahren positiv beeinflussen (19;30;33;40).

Kinder vor und zu Beginn der Pubertät

In Beobachtungsstudien werden bei Kindern häufig moderate bis starke KA (*Moderate to Vigorous Physical Activity*, MVPA) mittels Accelerometrie oder Fragebögen (Berechnung von KA-Skalen) erfasst (Tabellen 2a, 2b). Ergebnisse aus Querschnitts- und longitudinalen Untersuchungen zeigen einen positiven Zusammenhang zwischen MVPA und der KF (9-11;41;42). So wird bei Grundschulkindern die 40 Minuten am Tag moderat bis stark körperlich aktiv sind eine bis zu 3%-5% höhere KF erwartet als bei Kindern, die nur 10 Minuten MVPA am Tag durchführen (41). Teilweise wurde für moderate KA ein größerer osteogener Effekt auf die unteren Extremitäten ermittelt als für starke KA (42). Die mittels Accelerometrie gemessene MVPA erklärt einen höheren Varianzanteil der KF als der häufig eingesetzte Fragebogen *Physical Activity Questionnaire for Children* (9). Das deutet darauf

hin, dass aufgrund der durch Accelerometrie erfassten Beschleunigung in der vertikalen Bewegung der Effekt von WBA auf die KF besser festgehalten werden kann, als mithilfe von Fragebögen und errechneten KA-Skalen.

Gegenüber Beobachtungsstudien untersuchen Interventionsstudien die Wirkung definierter, häufig schulbasierter, Sport- und Bewegungsprogramme auf den Knochenzuwachs. Die über mehrere Monate durchgeführten Interventionsprogramme, häufig bestehend aus Sprungübungen (Seilspringen, Treppen-, Boxenhüpfen), erhöhten die KF an Lendenwirbelsäule, Hüfte und Oberschenkelhals (Tabelle 3).

Studien, die Kinder vor und zu Beginn der Pubertät einschlossen, zeigten, dass die KF durch MVPA und Sportprogramme vor der Pubertät stärker erhöht waren, als zu Beginn der Pubertät (11;12;30;43-45). Besonders bei präpubertären Jungen wurde in einigen Beobachtungs- und Interventionsstudien ein bedeutender osteogener Effekt von KA und Sport beobachtet (10;11;45;46). Das lässt sich durch eine stärkere Beziehung zwischen KA und der KF sowie durch die höhere KA/MVPA und Muskelstärke bei Jungen erklären (10;11;41;42;47). Aber auch bei Mädchen konnte vor der Pubertät ein nennenswerter Effekt von Sport auf die KF gezeigt werden, wobei diese Studien keine Jungen einschlossen und somit ein direkter Vergleich zwischen den Geschlechtern schwierig ist (48;49).

Schulbasierte Sportprogramme und KA hatten bei Mädchen auch zu Beginn der Pubertät noch einen positiven Effekt auf den Knochenzuwachs (16;50-54). Bei ihnen beobachteten einige Studien mit Eintritt in die Pubertät einen stärkeren Einfluss von KA und Sport als im präpubertären Alter (52;54). Dieser Effekt kann bei Mädchen, aber auch bei Jungen auf die erhöhte Östrogenproduktion zurückgeführt werden (21;24). Hingegen wurde für den ansteigenden Testosteronspiegel bei Jungen zu Beginn der Pubertät kein direkter Einfluss auf die Knochen, sondern lediglich auf die Muskelmasse gefunden (21).

Diese Ergebnisse deuten darauf hin, dass KA und Sport bei Mädchen und Jungen sowohl vor als auch zu Beginn der Pubertät für einen beschleunigten Knochenzuwachs von großer Bedeutung sind. Die in den RCTs durchgeführten schulbasierten Interventionsprogramme bestätigen die Annahme, dass verstärkter Sportunterricht in Schulen ausreicht, um bei den Kindern die KF zu erhöhen (40).

Jugendliche und junge Erwachsene

Bei Jugendlichen sowie bei jungen Erwachsenen (bis zum 20. Lebensjahr) wurden in Beobachtungsstudien weniger die allgemeine KA als vielmehr der Einfluss von WBA in Form bestimmter Sportarten auf den Knochenzuwachs untersucht. Vor allem im Rahmen von

Querschnittsuntersuchungen wurden Jugendliche und junge Erwachsene aus Sportvereinen, wie Fußball, Squash oder Gymnastik rekrutiert (Tabelle 2a). Die spontanen und abrupten Start- und Stopmomente in den Bewegungsabläufen solcher Sportarten erhöhen die GRF (24;55). Die hierfür erforderlichen maximalen kurzzeitigen und wiederholenden Muskelkontraktionen üben dabei eine hohe mechanische Belastung auf den jeweils belasteten Knochen aus (20). Deshalb zeigten Jugendliche und junge Erwachsene, die in ihrer Freizeit Sport im Tennis-, Fußball-, Basketball- oder Volleyballverein durchführten, eine höhere KF als Jugendliche oder junge Erwachsene ohne diese Freizeitsportarten (5;31;35;38;43;55-58). Dennoch wurde vor allem bei jungen Frauen eine Abnahme der Sensitivität der Knochen gegenüber KA und Sport beobachtet (58). Dieses Ergebnis wird durch Studien unterstützt, die alle drei Reifegrade, das heißt vor, zu Beginn und nach der Pubertät, einschlossen. Sowohl bei Jungen als auch bei Mädchen war ab dem 14. Lebensjahr die Wirkung von KA und Sport auf die KF geringer als davor (43;51;59;60). Ob diese Beobachtung auf den Abschluss der Pubertät und der Körperlängenentwicklung sowie auf die Beendigung des Wachstums der Gelenkflächen zurückzuführen ist, wurde in der Literatur bisher kaum untersucht. Auch existieren bislang nur wenige Interventionsstudien, die die osteogene Wirkung bestimmter Sportarten in der späten und nach der Pubertät sowie im jungen Erwachsenenalter (14.-20. Lebensjahr) untersuchen.

Nur wenige Studien haben die Effektivität von KA und Sport(-programmen) gemeinsam untersucht und miteinander verglichen. So ermittelten Meyer et al. für KA, erfasst mit Accelerometrie, in prä- und peripubertären Kindern keinen positiven Zusammenhang mit der KF. Hingegen konnten sie für ein schulbasiertes Sportprogramm in der Sportgruppe einen signifikanten Knochenzuwachs beobachten (12). Eine Aussage zur Dosis-Wirkungs-Beziehung von KA und Sport auf den Knochenzuwachs im Kindes- und Jugendalter ist aufgrund der heterogenen Datenlage problematisch. Dieser Überblicksartikel bestätigt die Aussage des U.S. Department of Health, dass vor allem WBA mit einer GRF über dem Dreifachen des Körpergewichts einen osteogenen Effekt auf Lendenwirbelsäule und Hüftknochen haben (61). Mindestens zehn Minuten Sport, vor allem WBA, an zwei bis drei Tagen die Woche über einen Mindestzeitraum von mehr als sechs bis sieben Monaten zeigen bei Kindern vor und zu Beginn der Pubertät eine positive Veränderung von Knochendichte und -struktur (Tabelle 3), (61;62). Dabei hatten WBA mit einer höheren GRF (Achtfache Körpergewicht) innerhalb eines kürzeren Interventionszeitraums eine osteogene Wirkung im Vergleich zu WBA mit einer geringeren GRF (3-5-fache des Körpergewichts) (Tabelle 3).

In Bezug auf die Sensitivität bestimmter Knochenregionen und Indikatoren in den jeweiligen Reifestadien, konnte bisher kein klarer Trend beobachtet werden (Tabellen 2a, 2b, 3). Das ergibt sich unter anderem aus der Problematik, dass bei Mehrpunktuntersuchungen, insbesondere im Kindes- und Jugendalter infolge von Wachstum, Reifung und sich verändernden Proportionen nicht exakt derselben Messpunkt erfasst wird, wie in der jeweils vorherigen Untersuchung (45). Zudem ist aufgrund der unterschiedlichen Methoden zur Erfassung der KF, ein Vergleich der Indikatoren untereinander schwierig (Tabellen 2a, 2b, 3).

Trotz der teilweise heterogenen Datenlage und der eingeschränkten Vergleichbarkeit unterschiedlicher Indikatoren und Erfassungsmethoden, kann zusammenfassend sowohl für MVPA als auch für Sport bei Mädchen und Jungen vor und zu Beginn der Pubertät eine positive Wirkung auf den Knochenzuwachs konstatiert werden.

Umstritten bleibt der Effekt von Leistungssport auf die KF. Sportler stellen eine selektierte Gruppe dar, die durch eine mögliche genetische Disposition, zum Beispiel mit einer ausgeprägteren Muskulatur, bereits für bestimmte Sportarten geeigneter sind. Hinzu kommt die Schwierigkeit für Leistungssportler geeignete Kontrollpersonen zu finden, um für genetische, hormonelle und ernährungsbedingte Kofaktoren zu kontrollieren (13;18;24;44). Um diesen Selektionsbias zu vermeiden, wird häufig an einem Individuum der Effekt mechanischer Belastungen auf die KF untersucht. So ist Tennis ein geeigneter Sport, um die KF des spielenden (belasteten) Arms mit der KF des nicht-spielenden (nicht belasteten) Arms zu vergleichen (35;43;63). Während Studien zu Tennis sowie zu anderen Sportarten einen positiven Effekt auf die belasteten Knochenregionen zeigten (5;13;31;35;38;43;44;55), wurde in anderen Untersuchungen an Leistungssportlern eine Verringerung der KF festgestellt. Auf der einen Seite bewirkt die reduzierte Verfügbarkeit von Energie für das Längenwachstum und für die Reifung einen verspäteten Eintritt in die Pubertät, vor allem bei weiblichen Leistungssportlern. Diese Wachstums- und Reifeverzögerungen sind stark mit einer verzögerten Knochenentwicklung assoziiert. Auf der anderen Seite erklärt sich die verringerte KF durch die sich wiederholenden, übermäßigen Belastungen des Knochens, wodurch es zur Ermüdung und Zerstörung der Mikrostruktur kommt. Abgebauter Knochen kann in Folge nicht schnell genug ersetzt werden und wird brüchig (19;24). Ob nach Einstellung des Leistungssports die zurückgebliebene Knochenentwicklung langfristig wieder aufgeholt werden kann, konnte bislang nicht ausreichend beantwortet werden (24;64).

Nachhaltigkeit

Die Nachhaltigkeit des osteogenen Effekts von KA und Sport im Kindes- und Jugendalter ist wenig untersucht. Häufig verfolgen Beobachtungs- und Interventionsstudien die Studienteilnehmer nur über wenige Jahre, so dass bisher keine Aussagen zu langfristig nachhaltigen Effekten über einen Zeitraum von zehn oder mehr Jahren gemacht werden können.

Allerdings wurde eine mittelfristig nachhaltige Wirkung von KA und Sport beobachtet. So zeigten Kinder vor der Pubertät noch drei bis fünf Jahre nach einem siebenmonatigen schulbasierten Sprungprogramm einen erhöhten Knochenmineralgehalt (*Bone Mineral Content*, BMC) (nach drei Jahren: 2.3%-4.4%; nach fünf Jahren: 1.4%), im Vergleich zur Kontrollgruppe (33). Ebenso bewirkte ein hohes Maß an KA im präpubertären Alter einen längerfristig nachhaltigen Effekt auf die KF im Vergleich zu präpubertären Kindern mit niedriger KA (4). Fünfjährige mit einer hohen MVPA hatten auch noch nach drei und sechs Jahren (das heißt im Alter von acht und elf Jahren) eine um 4%-14% höhere BMC (Ganzkörper, Hüfte, Lendenwirbelsäule), als fünfjährige Kinder mit einer geringen MVPA (10). Trotz der jährlichen Abnahme des nachhaltigen Effekts blieb dieser über den Beobachtungszeitraum statistisch signifikant (7;10;33). Auch im Jugend- und frühen Erwachsenenalter hatte Sport (Fußball, Eishockey) einen mittelfristig nachhaltigen Effekt auf die KF. Selbst fünf Jahre nach Beendigung des Trainings wurde bei männlichen Ex-Sportlern ein um 4%-8% höherer BMD festgestellt, als in der Kontrollgruppe (65). Ebenso konnte für junge Frauen, die während ihrer Jugendzeit sportlich aktiv waren (Fußball), noch acht Jahre nach Beendigung ein im Vergleich zur Kontrollgruppe höherer BMD am Oberschenkelhals nachgewiesen werden (58). Inwiefern diese Nachhaltigkeit von KA und Sport bis ins hohe Erwachsenenalter reicht, wird im nächsten Abschnitt diskutiert.

Einfluss KA auf die Knochengesundheit im Erwachsenenalter

Infolge eines veränderten Hormonspiegels im Erwachsenenalter nehmen nach Erreichen der PBM die Aufbau- und Umstrukturierungsprozesse im Knochen ab. Bei postmenopausalen Frauen finden kaum noch knochenaufbauende Prozesse statt (6). Die Knochenfläche nimmt ab dem 60. Lebensjahr altersbedingt ab (66). Der durchschnittliche altersbedingte Knochenschwund (BMC) im Jahr beträgt bei postmenopausalen Frauen in etwa 0.5% (67). Der Knochen verliert an Festigkeit. Damit erhöht sich das Risiko für Osteoporose und osteoporosebedingte Frakturen, insbesondere an Hüfte und Wirbelsäule (14;26;27;68). Inwiefern KA und Sport die knochenaufbauenden und Knochenumstrukturierungsprozesse im

prä- und postmenopausalen Alter unterstützen ist bis heute nicht eindeutig geklärt. Allerdings konnte sowohl in Beobachtungs- als auch in Interventionsstudien durch KA und Sport ein verringerter altersbedingter Knochenschwund an den unteren Extremitäten und Lendenwirbelsäule beobachtet werden (1;2;6;8;15;17;69-73). Zudem war sowohl im prä- als auch im postmenopausalen Alter die MVPA assoziiert mit einem reduzierten Risiko für Hüftfrakturen. So wurde für Frauen ein bis zu 38% und für Männer ein bis zu 45% reduziertes Hüftfrakturrisiko ermittelt (74).

Wie im Kindes- und Jugendalter, untersuchen Beobachtungs- und Interventionsstudien im Erwachsenenalter den Einfluss jeweils unterschiedlicher Arten körperlicher und sportlicher Aktivität auf die KF (Tabellen 4a, 4b, 5). Beobachtungsstudien richteten den Fokus sowohl auf die allgemeine KA in der Freizeit (häufig erfasst mit Fragebögen und KA-Skalen) als auch auf definierte Sportarten, wie Fußball, Squash oder Gymnastik. Allerdings wurde keine Studie gefunden, die einen möglichen osteogenen Effekt von MVPA (mittels Accelerometrie) sowohl für das prä- als auch für das postmenopausale Alter ermittelt. Interventionsstudien hingegen bewerten die osteogenen Wirkung von Sportprogrammen, häufig kombiniert aus aeroben (Ausdauersportarten, wie Laufen, schnelles Gehen) und anaeroben Aktivitäten (Resistenztraining) (Tabelle 5).

Prämenopausales Alter

Trotz unterschiedlicher Studiendesigns bestätigen Beobachtungs- und Interventionsstudien die positive Wirkung von WBA auf die KF an Lendenwirbelsäule, Hüfte, Oberschenkelhals- und Unterarmknochen (8;44;55;72;73;75;76).

Ein Vergleich der Interventionsergebnisse zeigt, dass Resistenztraining ohne zusätzliche aerobe Sportübungen und durchgeführt in einem Zeitraum von unter einem Jahr keine osteogene Wirkung hatte (77-79). Erst die Durchführung eines Resistenztraining an Ober- und Unterkörper, durchgeführt in einem Zeitraum von über zwölf Monaten, zeigte eine signifikante Zunahme des BMD an Lendenwirbelsäule und Trochanter (76). Resistenztraining in Kombination mit Lauf- und Sprungübungen, bewirkte nach zwölf Monaten ebenfalls eine Verbesserung der KF der unteren Extremitäten, nicht aber der Lendenwirbelsäule (8;72;75;76). Eine mögliche Erklärung ist, dass durch Laufen oder Sprungübungen primär die Knochen der unteren Extremitäten und Hüfte beansprucht werden, weniger die der Lendenwirbelsäule. Lediglich für Sport wie Fußball oder Squash konnten schwache osteogene Effekte an der Lendenwirbelsäule nachgewiesen werden. Diese erklären sich dadurch, dass diese Sportarten nicht nur die Muskeln der unteren Extremitäten, sondern die Muskeln des

gesamten Bewegungsapparates, auch die der Lendenwirbelsäule, intensiv beanspruchen (55;73).

Für Männer, in der Altersspanne von 20 Jahren bis zum Einsetzen der Andropause, wurde in der Literaturrecherche keine Studie gefunden, die den Zusammenhang zwischen körperlicher/sportlicher Aktivität und dem Knochenstatus untersucht.

Postmenopausales Alter

Weitaus mehr Studien existieren zum Einfluss von KA und Sport auf die KF bei Frauen im postmenopausalen Alter. Beobachtungsstudien zeigen, dass körperlich aktive Frauen eine höhere KF haben, als körperlich inaktive Frauen (6;15;80). Bereits schnelles Gehen (6.1 km/h) über eine Distanz von 4.8 km vier mal die Woche, bei über 82% der maximalen Herzfrequenz und einem Sauerstoffvolumen von 74% VO_2max verringerte den altersbedingten Knochenschwund (69). Unabhängig von der Geschwindigkeit, zeigten Frauen mit einer höheren Schrittzahl bessere Werte an der Lendenwirbelsäule als Frauen mit einer geringeren Schrittzahl (6). Regelmäßiges schnelles Gehen reduzierte zudem das Risiko für Hüftfrakturen (81).

Aus Interventionsstudien gehen unterschiedliche Ergebnisse zum osteogenen Effekt von Resistenztrainings und deren Kombination mit Lauf- und Sprungübungen hervor. Ein sechsmonatiges Resistenztraining, ohne zusätzliche Lauf- und Sprungübungen erzielte bei gesunden postmenopausalen Frauen mit Kalziumsupplementation keine signifikanten positiven Veränderungen der KF. Allerdings wurde in dieser Interventionsstudie keine Kontrollgruppe berücksichtigt, weshalb eine Aussage zu einem möglichen verringerten Knochenschwund durch das Resistenztrainings problematisch ist (77). Auch bei Frauen mit Osteopenie und Östrogendefizit bewirkte ein Resistenztraining über acht bis zwölf Monate keine osteogenen Effekte (82;83). Eine Ausnahme zeigte bei Frauen mit Östrogendefizit ein sechsmonatiges intensives Resistenztraining, bei denen sich der BMD an Trochanter und Oberschenkelhals erhöhte (71). Lediglich die Kombination von Resistenztraining mit Lauf- und Sprungübungen über einen Zeitraum von mindestens zehn Monaten wirkte bei gesunden und osteopenen Frauen einem altersbedingten Knochenschwund entgegen (2;70;84;85).

Die Frage nach der präventiven Wirkung von KA und Sport für Osteoporose ist bisher nicht eindeutig beantwortet. Sensible Knochen für Osteopenie, Osteoporose oder osteoporosebedingte Frakturen sind der Hüftknochen (insbesondere der Oberschenkelhals) oder die Lendenwirbelsäule (26;27). An diesen Knochenregionen wurde infolge von KA und kombinierten Sportprogrammen häufig kein osteogener Effekt beobachtet (Tabellen 4a, 4b, 5)

(2;69-71;77;80;82-86). So bewirkte ein bis zu drei Jahre durchgeführtes kombiniertes Sportprogramm bei osteopenen Frauen einen geringen bis keinen osteogenen Effekt an der Hüftregion (70;84). Auch an der Lendenwirbelsäule konnten Interventionsprogramme (kombiniert aus Resistenztraining und Schnellem Gehen) unter sechs Monaten keine bedeutenden positiven Veränderungen der KF erzielen (69;71). Erst die Kombination der Sportprogramme mit Kalziumsupplementation oder einer Hormon-Ersatz-Therapie (HRT) über einen Zeitraum von mindestens zehn Monaten hatte eine osteogene Wirkung an der Lendenwirbelsäule und am Trochanter (2;70;84). Aufgrund der zusätzlichen Kalziumsupplementation und HRT sind Aussagen über den osteogenen Effekt kombinierter Sportprogramme schwierig. Dennoch deuten diese Studienergebnisse darauf hin, dass ein protektiver Effekt für Osteoporose bei postmenopausalen Frauen, vor allem an der Lendenwirbelsäule, nur mithilfe kombinierter und langfristig durchgeführter Sportprogramme und -arten, die den gesamten Bewegungsapparat belasten, bewirkt werden kann (2;70;84). Die Wirkung am Oberschenkelhals bleibt fraglich (84;85;87). Trotz der geringen beobachteten osteogenen Effekte, sind langfristige Sportprogramme für das hohe Alter von großer Bedeutung. Die Kombination von WBA sowie Ausdauer-, Resistenz- und Balancetrainings verbessern die Muskelkraft, Balance und Koordination, wodurch zusätzlich das Sturzrisiko sowie sturzabhängige Frakturen bei postmenopausalen Frauen gesenkt werden konnten (6;8;74;88-91).

Andropause

Im Vergleich zu postmenopausalen Frauen haben Männer im hohen Alter eine geringere Prävalenz und Inzidenz von Osteoporose (92). Das kann unter anderem auf den allgemein höheren BMD zurückgeführt werden (2). Wie bei postmenopausalen Frauen zeigten Männer ab dem 50. Lebensjahr, die in der Freizeit noch körperlich und sportlich aktiv waren, eine bessere KF als körperlich inaktive Männer (1;3;6;93-95). Obwohl sich die positive Beziehung zwischen Freizeitsport und BMD (Hüfte) mit zunehmendem Alter abschwächte (3), hatte die Muskelkraft und -masse bei Männern mit zunehmendem Alter immer noch eine große Bedeutung für die KF (94;96). Der Zusammenhang zwischen KA / Sport und KF wurde bei Männern bisher überwiegend nur in Querschnittsstudien untersucht (Tabelle 4a). Die wenigen Interventionsstudien zeigen allerdings, dass Resistenztraining bei Männern während der Andropause bereits nach sechs Monaten einen osteogenen Effekt an der Lendenwirbelsäule hatte, während bei postmenopausalen Frauen ein Interventionseffekt erst nach zehn Monaten zu beobachten war (2;71;82). Allerdings beschreiben Whiteford et al., dass Resistenztraining keinen zusätzlichen Effekt auf den BMD bei älteren Männern hat. Durch schnelles Gehen

wurden dieselben Interventionseffekte erzielt (17). KA und Sport in der Freizeit schützten bei Männern im hohen Alter nicht nur vor Knochenschwund, sondern senkten auch das Risiko für Hüftfrakturen (74;97).

Wie im Kindes- und Jugendalter wurden auch im Erwachsenenalter verschiedene Methoden zur Erfassung der KF eingesetzt (Tabellen 4a, 4b, 5). Deshalb sind Aussagen zu einem Trend der Sensitivität der einzelnen Knochenparameter und Regionen sowie ein Vergleich dieser untereinander problematisch.

Klare Dosis-Wirkungs-Beziehungen von KA und Sport auf die KF bei Männern und Frauen sind aus bisherigen Metaanalysen nicht bekannt (61). Zusammenfassend lässt sich aber feststellen, dass kombinierte anaerobe und aerobe Sportarten (Resistenz-, Lauf- und Sprungübungen), für drei Tage pro Woche über mindestens zwölf Monate den altersbedingten Knochenschwund bei postmenopausalen Frauen reduzieren. Eine Kombination mit Kalziumsupplementation oder HRT kann die osteogene Wirkung dieser Sportprogramme erhöhen (2;84).

Nachhaltigkeit

Bisher untersuchten nur wenige longitudinale Studien den Einfluss von KA und Sport vom Kindes- und Jugendalter bis ins hohe Erwachsenenalter. Vor allem im Rahmen von Interventionsstudien wurden die Studienteilnehmer nur über wenige Jahre aktiv verfolgt. In Beobachtungsstudien erfolgte auch häufig nur eine retrospektive Querschnittsbefragung, in welcher die vergangene KA sowie die Durchführung bestimmter Sportarten bis ins Kindes- und Jugendalter zurück erfasst wurde (1;13;44;55;64;67;95;98). Allerdings zeigten die Studien unterschiedliche Ergebnisse. Kato et al. ermittelten bei Frauen (52-73 Jahre), die in ihrer Jugendzeit WBA betrieben, 40 Jahre später nachhaltige Veränderungen in BMC, Geometrie und Struktur am Oberschenkelhals (99). Demgegenüber reichte nach einer Untersuchung von Daly und Bass sportliche Aktivität im Jugendalter (13-18 Jahre) nicht aus, um unter anderem die Knochenstruktur und -festigkeit am Oberschenkelhals im hohen Alter (50-87 Jahre) aufrecht zu erhalten (95). Die größte osteogene Wirkung wurde infolge von kontinuierlich und aktuell durchgeführter körperlicher oder sportlicher Aktivität beobachtet (Tabellen 4b, 5). Bereits sechs bis zwölf Monate nach Abbruch des Trainings reduzierte sich bei prämenopausalen Frauen nicht nur die Muskelkraft, sondern auch der Interventionseffekt an Oberschenkelhals, Wirbelsäule und Gesamtkörper. Die Ex-Sportler zeigten sogar einen höheren Knochenschwund nach Abbruch der sportlichen Aktivität als die Kontrollen (75;76). Ein Abbruch des Trainings oder eine Verringerung regelmäßiger körperlicher und sportlicher

Aktivität in der Freizeit erhöhte zudem das Hüftfraktur- und Sturzrisiko (89). Ein langfristiger nachhaltiger Effekt von KA und Sport im Kindes- und Jugendalter auf das Frakturrisiko im hohen Erwachsenenalter wurde bisher nicht ermittelt (64;65).

Diese Studienergebnisse belegen, dass KA und Sport, vor allem WBA, kontinuierlich im Kindes-, Jugend- *und* Erwachsenenalter durchgeführt werden sollten, um einen protektiven und präventiven Effekt für die Knochengesundheit bis ins hohe Alter zu erreichen und Osteoporose vorzubeugen. Prospektive Langzeituntersuchungen, die die Nachhaltigkeit von KA und diversen Sportarten auf die Knochengesundheit bis ins hohe Erwachsenenalter untersuchen, sind bisher nicht bekannt (64;65;99).

Zusammenfassung

Dieser Überblicksartikel beschreibt den Einfluss körperlicher und sportlicher Aktivität auf den Knochenzuwachs und die Knochengesundheit in unterschiedlichen Lebensphasen. Beobachtungs- und Interventionsstudien konnten eine osteogene Wirkung von KA und Sport, vor allem von WBA bei Kindern, Jugendlichen und Erwachsenen bis ins hohe Alter belegen. Zudem wurde für hohe KA und Sport neben der osteogenen Wirkung, auch eine Erhöhung der Mager-, insbesondere der Muskelmasse und eine Verringerung des Fettgewebes beobachtet (16;42;46;57;94).

Im Kindesalter sind vor und zu Beginn der Pubertät bei Jungen und Mädchen sensible Phasen, in denen durch regelmäßige MVPA und Sport der Knochenzuwachs beschleunigt werden kann, der noch einige Jahre später Bestand hat. Im Erwachsenenalter nimmt der nachhaltige Effekt von KA und Sport mit zunehmendem Abstand zur sportlich aktiven Zeit ab. Deshalb kann das Risiko für Osteoporose und osteoporosebedingte Frakturen, insbesondere bei postmenopausalen Frauen nur durch regelmäßige mehrmonatige Sportprogramme, kombiniert aus Resistenz-, Lauf- und Sprungübungen reduziert werden. Zusätzliche HRT und Kalziumsupplementation können helfen das Risiko für Osteopenie, Osteoporose und osteoporosebedingte Frakturen zu verringern. Weitere Untersuchungen sind nötig, um auf einen therapeutischen Effekt sowohl von KA/MVPA und Sport bei postmenopausalen Frauen mit Östrogendefizit oder mit bereits bestehender Osteopenie/Osteoporose zu schließen.

Aussagen über eine langfristig nachhaltige Wirkung von KA und Sport sowohl auf die Erhöhung der KF als auch auf die Reduktion des Sturz- und Frakturrisikos im gesamten Lebenslauf, sind aufgrund des Mangels an prospektiven Langzeitstudien nur beschränkt möglich. Studienergebnisse weisen lediglich auf einen mittelfristigen Interventionseffekt von

Sport auf die KF in den jungen Lebensjahren hin. Da der überwiegende Anteil von Frakturen nicht nur auf eine geringe KF, sondern auch auf Stürze über die gesamte Altersspanne zurückzuführen ist, könnten in diesem Kontext die Zusammenhänge zwischen den neuromuskulären Funktionen und dem Sturzrisiko eine besondere Bedeutung haben. Diese Zusammenhänge fanden vor allem in der Literatur zur Wirkung der KA im Kindes- und Jugendalter bisher wenig Berücksichtigung. Die Bedeutung von Sport- und Bewegungsprogrammen im Kindes- und Jugendalter erklärt sich nicht nur aus der hohen osteogenen Wirkung von KA vor Abschluss des Längenwachstums und der Pubertät. Sport und Bewegungsprogramme verbessern auch die motorischen Fähigkeiten. Ob dadurch das Sturzrisiko und folglich das spätere Fraktur- und Osteoporoserisiko bereits im Kindes- und Jugendalter gesenkt werden kann, sollte Inhalt zukünftiger Studien sein.

Eine Limitation vieler Studien ist die Rekrutierung zu kleiner Stichproben. Zudem erschwert die geringe Datenlage zur nachhaltigen Wirkung von MVPA eine sichere Beantwortung der Frage, inwiefern dadurch das spätere Osteoporoserisiko vermindert wird. Weitere Langzeituntersuchungen, vornehmlich bevölkerungsbasierte RCTs mit größeren Stichproben, einer längeren Beobachtungszeit und standardisierten Studienprotokollen sind nötig, um eine langfristig nachhaltige Wirkung von KA und Sport auf die KF und das spätere Osteoporoserisiko nachzuweisen.

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Tabellenverzeichnis

Tabelle 1: Knochenregionen, KF-Indikatoren und Erfassungsmethoden zur Bestimmung der Knochenfestigkeit in Beobachtungs- und Interventionsstudien

Knochenregionen		KF-Indikatoren		Methoden zur Erfassung der KF	
TB	Ganzer Körper *(Total Body)*	BA	Knochenfläche (*Bone Area*)	DEXA	*Dual Energy X-ray Absorptiometry*
OE	Obere Extremitäten	BMC	Knochenmineralmasse (*Bone Mineral Content)*	HDR	*Dual Energy Radiograph Absorptiometry*
UE	Untere Extremitäten	BMD	Knochenmineraldichte (*Bone Mineral Density)*	HSA	*Hip Structure Analysis*
ARM	Arme	aBMD	Flächenmineraldichte (*Areal BMD*)	MRI	*Magnet Resonanz Imaging*
ARM^{dist}	Distale Seite vom Unterarm (*Distal Forearm*)	vBMD	Volumenmineraldichte (*Volumetric BMD)*	pQCT	*Peripheral Quantitative Computed Tomography*
LEG	Beine	BMAD	Berechneter BMD (*Bone Mineral Apparent Density*)	QUS	*Quantitative Ultrasound*
LS	Lendenwirbelsäule	CSA/ToA	Querschnittsfläche des Gesamtknochens		
HUM	Humerus	CSMI	*Cross-Sectional Moment of Inertia*		
HUM^{sh}	Humerusschaft (*Humerual Shaft)*	Cf/width	Knochenumfang (*Bone Circumference)*		
HUM^{prox}	Proximale Seite vom Humerus	BR	Ratio für Biegefähigkeit (*Buckling Ratio)*		
RAD	Radius	Z	*Section Modulus*		
RAD^{dia}	Radiusdiaphyse *(Radius Diaphyse)*	I_p	Knochenfestigkeit (*Bone Strength*)		
RAD^{dist}	Distale Seite des Radius *(Distal Radius)*	BSI	Knochenfestigkeit-Index (*Bone-Strength-Index)*		
RAD^{prox}	Proximale Seite vom Radius	SSI	Belastbarkeits-Index (*Strength-Strain-Index)*		
RAD^{sh}	Radius Schaft *(Radial Shaft*)	IBS	*Index of Bone Structural Strength*		
RAD^{UD}	Ultradistal Radius	Imin/Imax	*Moments of inertia (minimum/maximum)*		
UL	Ulna	CoA	Querschnittsfläche des kortikalen Knochens		
HIP	Ganzer Hüfteknochen (*Total Hip*)	CoTh	Kortikale Dicke (*Cortical Thickness)*		
WT	Wardsche Dreieck (*Ward Triangle*)	CoBMC	Kortikaler BMC (*Cortical BMC)*		
TR	Trochanter	CoBMD	Kortikaler BMD (*Cortical BMD)*		
IT	Intertrochanter	CovBMD	Korikaler vBMD (*Cortical vBMD)*		
F^{dia}	Diaphyse vom Femur	CWT	Dicke der kortikalen Knochenwand		
F^{dist}	Distale Seite vom Femur	TrA	Querschnittsfläche des trabekulären Knochen		
F^{mid}	Femur Schaftmitte (*Midfemur)*	TrTh	Trabekuläre Dicke (*Trabecular Thickness)*		
F^{sh}	Oberschenkelschaft (*Femoral Shaft)*	TrBMD	Trabekuläre BMD (*Trabecular BMD)*		
F^{total}	Ganzer Femur *(Total Femur)*	TrvBMD	Trabekulärer vBMD (*Trabecular vBMD*)		
F^{prox}	Proximale Seite vom Femur	SI	Knochensteifigkeitsindex (*Stiffness-Index*)		
FN	Obeschenkelhalsknochen *(Femoral Neck)*	VOS	Knochensteifigkeitsindex (*Stiffness-Index*)		
NN	*Narrow Neck*	SOS	Ultraschallgeschwindigkeit (*Speed of Sound)*		
TIB	Tibia	BUA	Ultraschallabschwächung (*Broadband Ultrasound Attenuation)*		
TIB^{dia}	Diaphyse der Tibia				
TIB^{dist}	Distale Seite der Tibia				
TIB^{prox}	Proximale Seite der Tibia				
TIB^{sh}	Schaft der Tibia (*Midschaft*)				

Tabelle 2a: Ergebnisse von Querschnittsstudien im Kindes- und Jugendalter

Studie	Studien-umfang	Altersspanne (Jahre)	Methode/Art KA/ Sport	Methode/ KF-Indikatoren[(a)]	Effektmaß		Bemerkung
Tobias et al., 2007 (42)	♂ und ♀ N: 4457	11.8	*Acc.* Moderate Aktivität (MPA, 100 counts/min) Starke Aktivität (VPA, 100 counts/min)	*DEXA*	MPA	VPA	- Multiple lineare Regression: AV=TB_{BMC}, TB_{BMD}, UE_{BMC}, UE_{BMD}, OE_{BMC}, OE_{BMD}, UV=MVPA - Die Knochenfläche der Knochenregionen war ebenfalls sig. postiv assoziiert mit MVPA
				TB_{BMC} (g)	β=18.83, *p<.001*	β=6.59, *p=.03*	
				TB_{BMD} (g/cm²)	β=7.48, *p<.001*	β=2.44, *p=.04*	
				UE_{BMC} (g)	β=13.07, *p<.001*	β=2.25, *NS*	
				UE_{BMD} (g/cm²)	β=12.61, *p<.001*	β=2.91, *NS*	
				OE_{BMC} (g)	β=0.11, *NS*	β=1.34, *p=.008*	
				OE_{BMD} (g/cm²)	β=0.17, *NS*	β=2.83, *p=.004*	
Janz et al., 2008 (9)	♂ und ♀ N: 449	11.2	*Acc.* MVPA (min/Tag)	*DEXA*			- Multivariate Regression: AV=HIP_{BMC}, LS_{BMC}, TB_{BMC}, UV=MVPA, adj. für KH, KG, TS - KA zusätzlich erfasst mit FB (PAQ-C), der eine geringere aufklärende Varianz zeigte als Acc.-Daten.
				♂ HIP_{BMC} (g)	β=0.04, *p<.001*		
				♂ LS_{BMC} (g)	β=0.03, *p<.001*		
				♂ TB_{BMC} (g)	β=1.42, *p<.001*		
				♀ HIP_{BMC} (g)	β=0.02, *p=.033*		
				♀ LS_{BMC} (g)	β <0.01, *NS*		
				♀ TB_{BMC} (g)	β=0.71, *p=.054*		
Kriemler et al., 2008 (11)	♂ und ♀ N: 449	6-13	*Acc.* VPA (min /Tag)	*DEXA*	♂	♀	- Partielle Korrelation, adj. für Alter, KH, TS - Keine sig. Ass. zwischen VPA und LS/TB - ♂ im niedrigsten VPA-Tertil hatten eine um 7.3% geringere HIP_{BMC} als ♂ im höchsten VPA-Tertil
				HIP_{BMC} (g)	r=0.24, *p<.01*	r=0.10, *NS*	
				HIP_{BMD} (g/cm²)	r=0.24, *p<.01*	r=0.14, *NS*	
Wang et al., 2005 (30)	♀ N: 242	PRÄ, Beginn PUB 10-12	*FB* KA_{hoch} (vs. $KA_{niedrig}$)	*DEXA, pQCT*	**PRÄ**	**Beginn PUB**	- Dieselben Trends zeigen sich für „hohe WBA“ vs. „keine/geringe WBA“
				TB_{BMC}	+5.2%, *p=.015*[(c)]	+1.2%, *NS*[(c)]	
				TB_{aBMD}	+2.9%, *p=.023*[(c)]	+0.6%, *NS*[(c)]	
				LS_{BMC}	+8.2%, *p=.025*[(c)]	+6.1%, *p=.054*[(c)]	
				LS_{aBMD}	+4.9%, *NS*[(c)]	+6.3%, *p=.025*[(c)]	
				F^{total}_{BMC}	+5.0%, *p=.016*[(c)]	+4.5%, *NS*[(c)]	
				F^{total}_{aBMD}	+5.6%, *p=.016*[(c)]	+4.1%, *NS*[(c)]	
				TIB^{sh}_{BMC}	+4.6%, *p<.001*[(c)]	+0.0%, *NS*[(c)]	
				TIB^{sh}_{vBMD}	+1.7%, *NS*[(c)]	+0.7%, *NS*[(c)]	
				TIB^{sh}_{CSA}	+2.2%, *p=.003*[(c)]	+0.0%, *NS*[(c)]	
				TIB^{sh}_{CovBMD}	+1.7%, *p=.038*[(c)]	+0.5%, *NS*[(c)]	
				TIB^{sh}_{CoTh}	+3.8%, *p=.021*[(c)]	+2.0%, *NS*[(c)]	
Weeks et al., 2010 (16)	♂ und ♀ N: 99	13-14	*FB* BPAQ-Score Vertikale Sprunghöhe (cm)	*DEXA, QUS*	♂	♀	- Korrelationsplots - BPAQ-Score zudem Vorhersagewert für FN_{CSMI}, LS_{BMC}, LS_{IBS}, TR_{BMC}, TB_{BMC} bei ♂ und FN_{BMAD}, TR_{BMC} bei ♀
				FN_{BMC} (g)	r=0.35, *p=.03*	r=0.32, *p=.02*	
				BUA (dB/MHz)	r=0.26, *p=.05*	r=0.17, *p=.11*	
				FN_{BMC} (g)	r=0.43, *p<.01*	r=0.18, *p=.14*	
				LS_{BMC} (g)	r=0.50, *p<.01*	r=0.23, *p=.08*	
				TB_{BMC} (g)	r=0.38, *p=.02*	r=0.02, *p=.45*	
				BUA (dB/MHz)	r=0.28, *p=.03*	r=0.07, *p=.31*	
Tournis et al., 2010 (49)	♀ N: 49	PRÄ 9-13	GYM	*pQCT*	**%-Differenz für GYM (vs. C)**		- Rekrutiert aus Leistungssportvereinen (mindestens 2 Jahre Trainingsdauer)
				TIB_{BMC} (mg)	+20.0%, *p<.001*[(c)]		
				TIB_{vBMD} (mg/cm³)	+7.2%, *p=.007*[(c)]		
				TIB_{CSA} (mm²)	+13.4%, *p=.001*[(c)]		
				TIB_{CoBMC} (mg)	+30.3%, *p<.001*		
				TIB_{CoCSA} (mm²)	+30.1%, *p<.001*		
				TIB_{CoTh} (mm)	+25.8%, *p<.001*		
				TIB_{CoSSIp} (mm³)	+31.5%, *p<.001*		

Studie	Studien-umfang	Altersspanne (Jahre)	Methode/Art KA/ Sport	Methode/ KF-Indikatoren[(a)]	Effektmaß			Bemerkung
Bass et al., 2002 (43)	♀ N: 47	PRÄ, Beginn PUB und POST 8-17	*Tennis* Belasteter (vs. nicht-belasteter) Arm	*DEXA, MRI*	**%-Differenz für belasteter Arm (vs. nicht belasteter Arm):**			- Tennisclub für mindestens 2 Jahre - Ähnliche Trends an HUM^{dist}
					PRÄ	**Beginn PUB**	**POST**	
				HUM_{CoBA}	+7.7%, *p<.001*	+11.9%, *p<.01*	+12.1%, *p<.001*	
				HUM_{Ip}	+11.3%, *p<.001*	+16.9%, *p<.01*	+17.0%, *p<.001*	
Ferry et al., 2010 (5)	♀ N: 73	15.9±2.0 – 16.3±1.2[(b)]	*SOC, SWIM*	*DEXA, HSA*	**%-Differenz für SOC (vs. SWIM)**			- rekrutiert aus Leistungssportvereinen (mindestens 6 Jahre Trainingsdauer) - SOC (vs. SWIM): höhere Werte für TR_{BMC}, TR_{BMD}, WT_{BMC}, WT_{BMD}, TIB_{BMC}, TIB_{BMD} (+11.11% bis +20.71%, *p<.00*[(c)])
				TB_{BMC}	+16.6%, *p<.001*[(c)]			
				TB_{BMD}	+12.6%, *p<.001*[(c)]			
				LS_{BMC}	+19.7%, *p<.001*[(c)]			
				LS_{BMD}	+11.5%, *p<.001*[(c)]			
				HIP_{BMC}	+21.5%, *p<.001*[(c)]			
				HIP_{BMD}	+17.9%, *p<.001*[(c)]			
				FN_{BMC}	+22.3%, *p<.001*[(c)]			
				FN_{BMD}	+16.7%, *p<.001*[(c)]			
				RAD/UL_{BMD}	+5.6%, *p<.03*[(c)]			
Duncan et al. 2002 (31)	♀ N: 75	POST 15-18	RUN, SWIM, RAD, TRI	*DEXA*	**%-Differenz für RUN (vs. C, SWIM, RAD)**			- rekrutiert aus Sportvereinen - keine sig. Unterschiede in ARM_{BMD} zwischen den einzelnen Gruppen - keine sig. Unterschiede zwischen RUN und TRI
				TB_{BMD}	+8.6% - +9.2%			
				LS_{BMD}	+12.2%			
				FN_{BMD}	+9.7% - +10%			
				LEG_{BMD}	+11% - +13.2%			
Pettersson et a., 2000 (57)	♀ N: 50	POST 17.4±0.8	JUMP, SOC	*DEXA*	**%-Differenz für JUMP (vs. C)**			- rekrutiert aus Leistungssportvereinen (mindestens 2 Jahre Training) - Knochenfläche stark assoziiert mit Muskelkraft
				TB_{BMD}/TB_{BMC}	+6.5% - +14.1%, *p<.05*[(c)]			
				LS_{BMD}/LS_{BMC}	+12.4% - +18.4%, *p<.05*[(c)]			
				$TIB^{dia}{}_{BMD}/ TIB^{dia}{}_{BMC}$	+14.4% - +16.4%, *p<.05*[(c)]			
					%-Differenz für SOC (vs. C)			
				FN_{BMD}/FN_{BMC}	+11.6% - +11.6%, *p<.05*[(c)]			
				TR_{BMD}/TR_{BMC}	+14.3% - +18.3%, *p<.05*[(c)]			
					%-Differenz für JUMP (vs. SOC)			
				TB_{BMD}	+6.0%, *p<.05*			
				LS_{BMD}	+10.3%, *p<.05*			
				HUM_{BMD}	+8.6%, *p<.05*			
McKay et al., 2011 (100)	♂ und ♀ N: 278	POST 15-20	*FB (PAQ-A)* belastende KA vs. nicht belastende KA	*pQCT*	**Aufklärende Varianzen von belastender KA**			- Linear, multivariate Regression: AV=Indikatoren der TIB, UV=belastende KA, adj. für KH, KG, Tibialänge, Kalzium Muskelfläche, Herkunft, TS
				$TIB_{Imin-max}$	10%-12%, *p<.001* (in ♂)			
				TIB_{ToA}	6%, *p=.003* (in ♂), 4%, *p=.011* (in ♀)			
				TIB_{TrBMD}	5%, *p=.004* (in ♀)			

Acc.: Accelerometer; **Adj.**: adjustiert; **Ass.**: Assoziation; **AV**: Abhängige Variable; **BPAQ:** *Bone-specific Physical Activity Questionnaire*; **C**: Kontrollen; **FB**: Fragebogen; **FM**: Fettmasse; **GYM**: Eliteturnerinnen; **JUMP**: Springen/Springseil; **KA**: Körperliche Aktivität; **KF**: Knochenfestigkeit; **KG**: Körpergewicht; **KH**: Körperhöhe; **RUN**: Laufen; **MM**: Magermasse; **MVPA**: Moderate bis starke KA; **PAQ-A/C**: *Physical Activity Questionnaire for Adults/Children*; **POST**: nach der Pubertät **PRÄ**: Vor der Pubertät; **PUB**: zu Beginn der Pubertät; **RAD**: Radfahren; **RUN**: Rennen; **sig.**: signifikant; **SOC**: Fußball; **SWIM**: Schwimmen; **TRI**: Triathlon **TS**: Tannerstadium; **UV**: Unabhängige Variable; **VPA**: Starke KA; **vs.**: versus; **WBA**: *Weight-Bearing Activities*

[(a)] Abkürzungsverzeichnis siehe Tabelle 1

[(b)] Altersspanne ermittelt aus den gemittelten Altern pro Gruppe

[(c)] prozentuale Effekte ermittelt aus den gemittelten Indikatoren der Knochenfestigkeit

Tabelle 2b: Ergebnisse von longitudinalen Studien im Kindes- und Jugendalter

Studie (Beobachtungdauer)	**Studien-umfang**	**Altersspanne (Jahre)**	**Methode/Art KA/ Sport**	**Methode/ KF-Indikatoren**[(a)]	**Effektmaß**	**Bemerkung**
Janz et al. 2007 (41) (3 Jahre)	♂ und ♀ N: 468	5.3 (T0)	*Acc.* MVPA (10min/Tag)	*DEXA, HSA* FN_{CSA} (cm^2) FN_Z (cm^3)	β=0.012, *p<.001 (*in ♂) β=0.004, *p<.05* (in ♂)	- Mehrebenen-Regressionsanalyse, AV= FN_{CSA}, FN_Z, UV=MVPA, adj. für Alter, KH, KG - Keine sig. positive Ass. zwischen MVPA und FN_{CSA}/FN_Z bei ♀ - k.A. zu Alter
Janz et al., 2010 (10) (6 Jahre)	♂ und ♀ N: 333	5.3 (T0)	*Acc.* MVPA mit 5 J (10min/Tag)	*DEXA* TB_{BMC} (g) LS_{BMC} (g) HIP_{BMC} (g)	β=5.77, *p<.046 13* β=0.44, *p<.002* (in ♂) β=0.13, *p<.039 (*in ♂)	- Gemischtes lineares Regressionsmodell: AV=TB_{BMC}, LS_{BMC}, HIP_{BMC} mit 8 und 11 J; UV= MVPA mit 5 J, adj. für Alter, TS, KH, KG, aktuelle MVPA, BMC (5 J) - Keine sig. positive Ass. zwischen MVPA und BMC bei ♀
Devlin et al., 2002 (4) (6 Jahre)	♀ N: 78	11.9 (T0)	*FB* KA-Score Einteilung in Tertile	*DEXA, HSA* NN_{SI} IT_{SI}	**KA_{T3} (vs. KA_{T1} und KA_{T2})** +10-11%, *p<0.02* +10-11%, *p<0.05*	- KA erfasst ab dem 12. Lebensjahr - Erfassung der KF im Alter von 17 J
Sundberg et al., 2002 (60) (3 Jahre)	♂ und ♀ N: 72	PRÄ, Beginn PUB 13.2±0.3 (T0)	*FB* KA im Alter von 9-13J (Querschnittsbefragung) Einteilung in hohe (KA+) vs. niedrige (KA-)	*DEXA, QUS* FN_{BMC} FN_{aBMD} FN_{vBMD} BUA SOS SI	**KA+ (vs. KA-) zu T0** +15.1%, *p=.02* +13.0%, *p=.002* +11.6%, *p=.004* +7.6%, *p=.03* +1.5%, *p=.01* +13.0%, *p=.01*	- 3 J mehr KA+ oder KA- (zwischen 13-16 J), zeigte keine sig. erhöhten Unterschiede zwischen beiden KA-Gruppen - Sport und KA hatten zusätzliche Effekte vor dem 13. Lebensjahr, danach nicht mehr
Kontulainen et al., 2001 (35) (5 Jahre)	♀ N: 91	JUNG: 10.5±2.2 (Trainingsstart vor Menarche) ALT: 26.4±8.0 (Trainingsstart nach Menarche)	Tennis, Squash Belasteter vs. nicht-belasteter Arm	*DEXA* HUM^{prox}_{BMC} (JUNG) HUM^{sh}_{BMC} (JUNG) RAD^{dist}_{BMC} (JUNG) HUM^{prox}_{BMC} (ALT) HUM^{sh}_{BMC} (ALT) RAD^{dist}_{BMC} (ALT)	**Mittlere Seitenveränderung (%) zwischen belastetem und nicht belastem Arm von T0-T1** -2.7%[(d)] +0.3%, *NS* -2.1%[(d)] +2.3%[(d)] +1.3%[(d)] -0.4%, *NS*	- Rekrutiert aus Leistungssportvereinen - JUNG: verringerte Differenz von T0-T1, da Erhöhung des BMC im nicht belastetem Arm - ALT: erhöhte Differenz zwischen T0-T1, da Verringerung des BMC im nicht belastetem Arm und gleich bleibender BMC im belastetem Arm

Acc.: Accelerometer; **Adj.**: adjustiert; **Ass.**: Assoziation; **AV**: Abhängige Variable; **C**: Kontrollen; **FB**: Fragebogen; **J**: Jahre (Alter); **k.A.**: keine Angabe; **KA**: Körperliche Aktivität; **KA_{T1}**: 1. Tertile von KA; **KA_{T2}**: 2. Tertile von KA; **KA_{T3}**: 3. Tertile von KA; **KF**: Knochenfestigkeit; **KG**: Körpergewicht; **KH**: Körperhöhe; **MM**: Magermasse; **MVPA**: Moderate bis starke KA; **NS**: nicht signifikant; **PRÄ**: vor der Pubertät; **PUB**: zu Beginn der Pubertät; **sig.**: signifikant; **T0**: Basiserhebung; **T1**: 1. Follow-up; **TS**: Tannerstadium; **UV**: Unabhängige Variable

[(a)] Abkürzungsverzeichnis siehe Tabelle 1

[(d)] signifikant im 95%-Konfidenzintervall

Tabelle 3: Interventionsstudien (RCT, CT) im Kindes- und Jugendalter

Studie	Studien-umfang	Altersspanne (Jahre)	Methode/Art KA/ Sport	Methode/ KF-Indikatoren [(a)]	Interventionseffekte	Bemerkung
Fuchs et al., 2001 (32)	♂ und ♀ N: 89	PRÄ 5.9-9.8	7 Monate, 3x/W / Sprungprogramm - 10 min Springen/Session - 50-100 Sprünge/Session - GRF: 8,5xKG	*DEXA* FN_{BMC} FN_{BMD} FN_{BA} LS_{BMC} LS_{BMD}	**%-Differenz für I (vs. C)** +4.5% (k.A. zu *p*) +1.4%, *p=.085* +2.9% (k.A. zu *p*) +3.1% (k.A. zu *p*) +2.0% (k.A. zu *p*)	- Schulbasiert
Gunter et al., 2008a (33)	♂ und ♀ N: 57	PRÄ 7.6±1.0 – 7.9±1.0 [(b)]	7 Monate, 3x/W / Sprungprogramm - 10 min Springen/Session - 50-100 Sprünge/Session - GRF: 8,5xKG	*DEXA* HIP_{BMC}	**%-Differenz für I (vs. C)** +3.6%, *p<.05*	- Schulbasiert - I-Effekt verliert nach 3 Jahren an Bedeutung - 5 Jahre nach Interventionsende: HIP_{BMC}=1.4% - Keine I-Effekte an FN_{BMC} und TR_{BMC}
Linden et al., 2006 (48)	♀ N: 99	PRÄ 7-9	2 Jahre, zusätzlich 40min pro Tag extra WBA - Innen- und Außenaktivitäten (Lauf-, Spring-, Ball-, Kletterspiele)	*DEXA* LS^{2-4}_{BMC} LS^{2-4}_{aBMD} LS^{3}_{BMC} LS^{3}_{aBMD} LS^{3}_{width} LEG_{BMC} LEG_{aBMD} TB_{aBMD} FN_{width}	**Erhöhter jährlicher Zuwachs (%) für I (vs. C)** +3.8%, *p=.007* +1.2%, *p=.02* +7.2%, *p<.001* +1.6%, *p=.006* +1.8%, *p<.001* +3.0%, *p=.07* +1.2%, *p=.007* +0.6%, *p=.006* +0.3%, *p=.02*	- Schulbasiert
Valdimarsson et al., 2006 (40)	♀ N: 103	PRÄ 7-9	1 Jahr, 2-5x/W / Erhöhter Sportunterricht I: 40min Sport / 5xW C: 60min Sport / 1-2x/W	*DEXA* LS^{2-4}_{BMC} LS^{3}_{BMC} LS^{2-4}_{aBMD} LS^{3}_{aBMD} LS^{3}_{width}	**%-Differenz für I (vs. C)** +4.7%, *p<.001* +9.5%, *p<.001* +2.8%, *p<.001* +3.1%, *p<.001* +2.9%, *p<.001*	- Schulbasiert - Nicht randomisiert
Gunter et al., 2008b (7)	♂ und ♀ N: 205	PRÄ 8.6±0.9	7 Monate, 3x/W / Sprungprogramm - 10 min Springen/Session - 50-100 Sprünge/Session - GRF: 8,5xKG	*DEXA* FN_{BMC} LS_{BMC} HIP_{BMC} TB_{BMC}	**%-Differenz für I (vs. C)** +7.7%, *p<.05* +7.9%, *p<.05* +8.4%, *p<.05* +7.3%, *p<.05*	- Schulbasiert - I (vs. C) 3 Jahre nach Interventionsende: FN_{BMC} 4.4%, *p<.05*, LS_{BMC} 2.3%, *p<.05*, HIP_{BMC} 3.2%, *p<.05*, TB_{BMC} 2.9%, *p<.05*
MacKelvie et al., 2004 (46)	♂ N: 64	PRÄ 10.1±0.5 – 10.2±0.5 [(b)]	20 Monate, 3x/W / Sprungprogramm - 10-12 min Springen/Session - 50-100 Sprünge/Session - GRF: 3,5-5xKG	*DEXA, HSA* FN_{BMC} NN_{CSMI} NN_{Z} NN_{CSA}	**%-Differenz für I (vs. C)** +4.3%, *p<.01* +12.4%, *p<.05* +7.4%, *p<.05* +2.5%, *p=.17*	- Schulbasiert - höherer Zuwachs für FN_{BA}, IT_{CSA} und IT_{CoTh} in I-gruppe (vs. C)
MacKelvie et al., 2001 [1] (52)	♀ N: 70	PRÄ 10.0±0.6 – 10.1±0.5 [(b)]	7 Monate, 3x/W / Sprungprogramm - 10-12 min Springen/Session - 50-100 Sprünge/Session - GRF: 3,5-5xKG	*DEXA*	Keine sig. Veränderungen der Indikatoren der KF an FN, TB, TR und LS	- Schulbasiert
Petit et al., 2002 [1] (54)	♀ N: 70	PRÄ 10.0±0.6 – 10.1±0.5 [(b)]	7 Monate, 3x/W / Sprungprogramm - 10-12 min Springen/Session - 50-100 Sprünge/Session - GRF: 3,5-5xKG	*DEXA, HSA*	Keine sig. Veränderungen der Indikatoren der KF und Knochenstruktur an FN, NN, IT und F^{sh}	- Schulbasiert

Studie	Studien-umfang	Altersspanne (Jahre)	Methode/Art KA/ Sport	Methode/ KF-Indikatoren [(a)]	Interventionseffekte	Bemerkung
McKay et al., 2000 (52)	♂ und ♀ N: 144	PRÄ, PUB 6.9-10.2	8 Monate, 3x/W / Sprungprogramm - 10 min Belastung /Session	*DEXA* TR_{aBMD}	**%-Differenz für I (vs. C)** +1.2%, *p<.05*	- Schulbasiert - Keine I-Effekte an FN, LS, TB und F^{prox}
MacKelvie et al., 2003 (53)	♀ N: 75	PRÄ, PUB 9.9±0.6 – 10.3±0.4 [(b)]	20 Monate, 3x/W / Sprungprogramm - 10-12 min Springen/Session - 50-100 Sprünge/Session - GRF: 3,5-5xKG	*DEXA* LS_{BMC} FN_{BMC}	**%-Differenz für I (vs. C)** +3.7%, *p<.05* +4.6%, *p<.05*	- Schulbasiert - Keine I-Effekt an TR und TB
Macdonald et al., 2007 (45)	♂ und ♀ N: 410	PRÄ, Beginn PUB 10.2±0.6	16 Monate, täglich / Sprungprogramm - 15 min Springen/Session	*pQCT* TIB_{BSI} TIB_{BMD}	**Absolute Differenz für I (vs. C):** +222.9mg²/mm⁴, (k.A. zu *p)* (nur in PRÄ ♂) +6.5 mg/cm³, (k.A. zu *p)* (nur in PRÄ ♂)	- Schulbasiert
Meyer et al., 2011 (12)	♂ und ♀ N: 502	PRÄ: 6-7 PUB: 11-12	9 Monate, 5x/W / Sprungprogramm + 2 zusätzliche Sportstunden - 15 min Springen/Session	*DEXA* FN_{BMC} LS_{BMC} LS_{BMD} TB_{BMC} TB_{BMD}	**%-Veränderung T0-T1 für I-Gruppe**: +5.4%, *p=.04* +4.7%, *p=.01* +7.3%, *p≤.001* +5.5%, *p=.001* + 8.4%, *p≤.001*	- Schulbasiert - Höhere I-Effekte bei PRÄ an TB_{BMC}, FN_{BMC}, LS_{BMC}
MacKelvie et al., 2001 [2] (52)	♀ N: 107	PUB 10.4±0.7 – 10.5±0.6 [(b)]	7 Monate, 3x/W / Sprungprogramm - 10-12 min Springen/Session - 50-100 Sprünge/Session - GRF: 3,5-5xKG	*DEXA* FN_{BMC} FN_{aBMD} FN_{vBMD} LS_{BMC} LS_{aBMD}	**%-Differenz für I (vs. C)** +1.9%, *p<.05* +1.6%, *p<.05* +3.1%, *p<.05* +1.8%, *p<.05* +1.7%, *p<.05*	- Schulbasiert
Petit et al., 2002 [2] (54)	♀ N: 107	PUB 10.4±0.7 – 10.5±0.6 [(b)]	7 Monate, 3x/W / Sprungprogramm - 10-12 min Springen/Session - 50-100 Sprünge/Session - GRF: 3,5-5xKG	*DEXA, HSA* FN_{BMD} FN_{CSA} FN_{CoTh} FN_{Z} IT_{BMD}	**%-Differenz für I (vs. C)** +2.6%, *p=.03* +2.3%, *p=.04* +3.2%, *p=.03* +4.0%, *p=.04* +1.7%, *p=.02*	- Schulbasiert
Weeks et al., 2008 (16)	♂ und ♀ N: 99	PUB, POST 13.8±0.4	8 Monate, 2x/W / Sprungprogramm - 10 min Springen/Session - ~300 Sprünge / Session	*DEXA, QUS* FN_{BMC} LS_{BMAD} TB_{BMC} FN_{BA} BUA	**%-Veränderung T0-T1 für I (vs. C)** +13.9%, *p=.05* vs. +4.9%, *NS* (nur in ♀) +5.2%, *p=.04* vs. +1.5%, *NS* (nur in ♀) +10.6%, *p=.001* vs. +6.3%, *p=.001* (nur in ♂) +3.8%, *p=.001* vs. +2.7%, *NS* (nur in ♂) +5.0%, *p=.01* vs. +1.4%, *NS* (nur in ♂)	- Schulbasiert - ♂ der I-Gruppe zeigten höhere TR_{BMC}, LS_{BMC} und TB_{BMC} als ♀ der I-Gruppe
Johannsen et al., 2003 (51)	♂ und ♀ N: 54	PRÄ: 3-8 PUB: 11-12 POST: 15-18	12 Wochen, 5x/W / Sprungprogramm - ~45 Sprünge/Session - GRF: 4xKG	*DEXA, pQCT* TB_{BMC} LEG_{BMC}	**%-Differenz für I (vs. C)** +34.7%, *p=.03* +41.9%, *p=.03*	- Nicht schulbasiert, Kinder rekrutiert im Tageszentrum - Nur in PUB zeigte Sprungprogramm I-Effekt an TIB^{dist}_{BMC}, TIB^{dist}_{vBMD}, LS_{BMC}

C: Kontrollen; **CT**: Kontrollierte Studie (nicht randomisiert); **GRF:** Bodenreaktionskraft (*Ground Reaction Force*); **I**: Intervention; **k.A.**: Keine Angabe; **KF**: Knochenfestigkeit; **KG**: Körpergewicht; **PRÄ**: Vor der Pubertät; **PUB**: Beginn Pubertät; **POST**: Späte/ Ende Pubertät; **RCT**: Randomisierte kontrollierte Studie; **sig.**: signifikant; **T0**: Basiserhebung; **T1**: 1. Follow-up; **WBA**: *Weight-Bearing Activities*; **x/W**: Anzahl pro Woche

[(a)] Abkürzungsverzeichnis siehe Tabelle 1

[(b)] Altersspanne ermittelt aus den gemittelten Altern pro Gruppe

Tabelle 4a: Ergebnisse von Querschnittsstudien im Erwachsenenalter

Studie	Studien-umfang	Altersspanne (Jahre)	Methode/Art KA/ Sport	Methode/ KF-Indikatoren[(a)]	Effektmaß	Bemerkung
Creighton et al., 2001 (38)	♀ N: 41	PRÄ 18-26	HI (Basketball, Volleyball) OI (Fußball, Laufen) NI (Schwimmen)	*DEXA* TB_{BMD} FN_{BMD} TR_{BMD} TB_{BMD} TR_{BMD}	**HI (vs. NI, C)** +14.3% - +16.9%, *p<.05*[(c)] +17.3% - +18.9%, *p<.05*[(c)] +18.1% - +19.0%, *p<.05*[(c)] **OI (vs. NI, C)** +6.7% - +8.9%, *p<.05*[(c)] +14.9% - +15.8%, *p<.05*[(c)]	- Rekrutiert aus Leistungssportvereinen
Heinonen et al., 2002 (44)	♀ N: 28	PRÄ 22.6-30.6[(b)]	GEW	*pQCT* F^{dist}_{BMD} RAD^{dist}_{CoA} RAD^{dist}_{BSI} RAD^{sh}_{CoA} RAD^{sh}_{CWT} RAD^{sh}_{BSI} TIB^{sh}_{CoA}	**%-Differenz für GEW (vs. C)** +11%, *p=.04* +38%, *p=.03* +41%, *p<.01* +26%, *p<.01* +13%, *p=.04* +43%, *p<.01* +9%, *p=.03*	- Rekrutiert aus Leistungssportvereinen (6.5 bis 14 Jahre Trainingsdauer)
Nikander et al., 2009 (55)	♀ N: 111	PRÄ 19.7(±2.4) – 28.9 (±5.6)[(b)]	HI (Drei-, Hochsprung) OI (Fußball, Squash) HM (Gewichtheben) LI (Ausdauerlauf) NI (Schwimmen)	*DEXA, MRI, pQCT* LS_{BMD} FN_{BMD} FN_{CoA} FN_{Z}	**%-Differenz für HI und OI (vs. C)** **HI (vs. C)** **OI (vs. C)** +25%, *p<.001* +15%, *p=.001* +30%, *p<.001* +20%, *p<.001* +30%, *p=.001* +30%, *p=.018*	- Rekrutiert aus Leistungssportvereinen - Keine sig. Unterschiede zwischen den Gruppen der KF am Radius
Nikander et al., 2010a (13)	♀ N: 254	PRÄ 20.2(±2.6) – 28.9 (±5.6)[(b)]	HI (Drei-, Hochsprung) OI (Fußball, Squash) HM (Gewichtheben) LI (Ausdauerlauf) NI (Schwimmen)	*pQCT* TIB^{dist}_{BMC} TIB^{dist}_{CoA} TIB^{dist}_{BSI} TIB^{sh}_{BMC} TIB^{sh}_{ToA} TIB^{sh}_{CoA} TIB^{sh}_{BSI}	**%-Differenz für HI, OI, LI (vs. C)** +15% - +26%, *p<.05* +33% - +53%, *p<.05* +20% - +46%, *p<.05* +28% - +44%, *p<.05* (nicht für LI) +13% - +21%, *p<.05* +18% - +28%, *p<.05* +19% - +32%, *p<.05*	- Rekrutiert aus Leistungssportvereinen
Uusi-Rasi et al., 2002 (98)	♀ N: 218	PRÄ: 32.6±2.2 POST:67.3±2	*Interview* Allgemeine KA (Kindes- und Erwachsenenalter) KA+ (starke KA) KA- (moderate, geringe KA)	*pQCT* RAD^{dist}_{BSI} RAD^{sh}_{BSI} TIB^{dist}_{BMC} TIB^{dist}_{TrBMD} TIB^{sh}_{BMC} TIB^{sh}_{CoA} TIB^{sh}_{BSI}	**%-Differenz für KA+ (vs. KA-)** **PRÄ** **POST** -8.1%[(d)] +13.4%[(d)] +4.4% (NS) +8.5%[(d)] -- +5.0%[(d)] +6.9%[(c)] -- +4.1% (NS) +5.9%[(d)] +4.2% (NS) +6.3%[(d)] -- +8.6%[(d)]	- Kalziumzufuhr positiv assoziiert mit Knochenstruktur des Radius, aber nicht Tibia in PRÄ und POST
Gerdhem et al., 2003 (101)	♀ N: 995	POST 75	*FB* Allgemeine KA	*DEXA* TB_{BMD}, LS_{BMD}, FN_{BMD}, TR_{BMD}	Keine sig. Assoziation zwischen KA und BMD-Werte	
Bleicher et al., 2010 (3)	♂ N: 1705	POST 70-97	*FB (PASE)* PASE-Score KA/ Gehen (km/Tag);	*DEXA* HIP_{BMD} (g/cm²) HIP_{BMD} (g/cm²)	β=1.4, *p<.001* β=1.4, *p<.001*	- Multivariate Regression: AV=BMD, UV=KA, adj. für Alter, KG

Studie	Studien-umfang	Altersspanne (Jahre)	Methode/Art KA/ Sport	Methode/ KF-Indikatoren[(a)]	Effektmaß	Bemerkung
					Sport (vs. kein Sport in d. Freizeit)	
			Sportaktivitäten in Freizeit	FN_{BMD} (g/cm^2)	6% (k.A. zu *p*)	
				HIP_{BMD} (g/cm^2)	8% (k.A. zu *p*)	
				LS_{BMD} (g/cm^2)	9% (k.A. zu *p*)	
Cousin et al., 2010 (94)	♂ N: 1171	POST 77.2±5.1	*FB (PASE)* Sport- /Freizeitaktivitäten eingeteilt in KA_{Q1}-KA_{Q4}	*DEXA, QCT*	**%-Differenz für KA_{Q4} (vs. KA_{Q1})**	
				TIB_{TrBSI}	+7%, *p=.025*	
				TIB_{TrToA}	+3%, *p=.008*	
				TIB_{TrSSI}	+4%, *p=.025*	
				TIB_{CoA}	+4%, *p=.006*	
				TIB_{CoBMD}	+1%, *p=.004*	
				TIB_{CoSSI}	+4%, *p=.014*	
				TIB_{CoZ}	+4%, *p=.006*	
Cauley et al., 2005 (93)	♂ N: 5995	POST 73.7±5.9	*FB (PASE)* KA (PASE-Score)	*DEXA* FN_{BMD} (g/cm^2)	β=0.97[(d)]	- Multivariate Regression: AV=FN_{BMD}, LS_{BMD}, HIP_{BMD} UV=KA, adj. für Alter, KG - Keine sig. Veränderungen für LS_{BMD} und HIP_{BMD}
Daly und Bass, 2006 (95)	♂ N: 161	POST 50-87	*FB* Sport/ KA in der Freizeit: Einteilung in „high impact" (H) und „low/non impact" (L) pro Altersstufe - H/H: H-Aktivitäten mit 13-18 / 19-50+ J - H/L: H-Aktivitäten mit 13-18 J - L/L: L-Aktivitäten mit 13-18 / 19-50+ J	*DEXA, QCT, QUS*	**%-Differenz für H/H (vs. L/L)**	- Selbstberichtete KA im Alter von 13-18 J ist kein signifikanter Prädiktor für spätere Knochenstruktur und Knochensteifigkeit
				F^{mid}_{ToA}	+6.1%, *p<.05*	
				F^{mid}_{CoA}	+6.6%, *p<.05*	
				F^{mid}_{CoBMC}	+6.7%, *p<.05*	
				F^{mid}_{Ip}	+12.4%, *p<.05*	
				VOS	+0.6%, *p<.05*	
				BUA	+8.2%, *p<.05*	
				HIP_{aBMD}	+6.6%, *p<.01* (H/H>H/L)	
Bailey et al., 2010 (1)	♂ N: 281	POST 50-79	*FB* Sport/ KA in der Freizeit Jugend- vs. Erwachsenenalter - H/H vs. H/L vs. L/L (vgl. Daly und Bass, 2006)	*QCT*	**%-Differenz für H/H (vs. L/L)**	- Für BMC Imin und Imax gilt: HH>HL>LL
				F^{mid}_{BMC}	+7.1%, *p<.01*	
				F^{mid}_{Imax}	+12.6%, *p<.01*	
				F^{mid}_{Imin}	+13.0%, *p<.001*	
				TIB^{mid}_{BMC}	+9.5%, *p<.001*	
				TIB^{mid}_{Imax}	+16.2%, *p<.001*	
				TIB^{mid}_{Imin}	+14.7%, *p<.001*	

C: Kontrollgruppe; **FB**: Fragebogen; **GEW**: Gewichtheben; **HI**: Hohe Belastung (*high impact*); **HM**: Hoher Kraftaufwand (*high magnitude*); **J**: Jahre; **KA**: Körperliche Aktivität; **KF**: Knochenfestigkeit; **LI**: Wiederholende, geringe Belastung (*repetitive low impact*); **NI**: Wiederholende, keine Belastung (*repetitive non-impact)*; **NS**: nicht signifikant; **OI**: Ungewohnte Belastung (*odd impact*); **PASE***: Physical Activity Summary Scale for Elderly*; **PRÄ**: vor Eintritt der Menopause; **POST**: während / nach Eintritt der Menopause; **Q_1-Q_4**: Quartile; **sig.**: signifikant

[(a)] Abkürzungsverzeichnis siehe Tabelle 1

[(b)] Altersspanne ermittelt aus den gemittelten Altern pro Gruppe[j]

[(c)] prozentuale Effekte ermittelt aus den gemittelten Indikatoren der Knochenfestigkeit

[(d)] signifikant im 95%-Konfidenzintervall

Tabelle 4b: Ergebnisse aus longitudinalen Studien im Erwachsenenalter

Studie (Beobachtungsdauer)	**Studien-umfang**	**Altersspanne (Jahre)**	**Methode/Art KA/ Sport**	**Methode/ KF-Indikatoren** [(a)]	**Effektmaß**			**Bemerkung**
Nordström et al., 2005 (65) (5 Jahre)	♂ N: 145	PRÄ 21.0±4.5 – 22.4 ±6.3[(b)]	Eishockey, Fußball Einteilung in Sportler und Ex-Sportler	*DEXA*	**%-Veränderung des BMD (T0-T1)**			- Rekrutiert aus Leistungssportvereinen - Zu T0 und T1 hatten Sportler und Ex-Sportler höhere BMD-Werte als Kontrollen - k.A. der Signifikanz für die %-Veränderung der jeweiligen Gruppen zwischen T0-T1
					Sportler	**Ex-Sportler**	**C**	
				TB_{BMD}	+3.7%[(c)]	+1.5%[(c)]	-3.3%[(c)]	
				LS_{BMD}	+4.5%[(c)]	+2.4%[(c)]	-7.0%[(c)]	
				FN_{BMD}	-1.5%[(c)]	-6.7%[(c)]	+5.1%[(c)]	
				ARM_{BMD}	+20.8%[(c)]	+14.2%[(c)]	-2.0%[(c)]	
Uusi-Rasi et al., 2008 (15) (10 Jahre)	♀ N: 267	PRÄ: 25-30 POST: 60-65	*Interview* Allg. KA (Kindes- und Erwachsenenalter) KA+ (starke KA) KA- (moderate, geringe KA)	*DEXA, HSA*	**%-Differenz der Veränderung T0-T1 für KA+ (vs. KA-)**			
					PRÄ	**POST**		
				FN_{BMC}	+3.8% (NS)	+6.9%[(d)]		
				TR_{BMC}	+6.7%[(d)]	+5.5%[(d)]		
				NN_{CSA}	+3.7% (NS)	+6.8%[(d)]		
				NN_{Z}	+2.0% (NS)	+9.6%[(d)]		
Uusi-Rasi et al., 2006 (67) (6 Jahre)	♀ N: 217	POST 62.0±4.6	GYM	*DEXA, pQCT*	**%-Veränderung zwischen T0-T1**			- Rekrutierung von Freizeitsportlerinnen (mindestens 34 Jahre Trainingsdauer)
					GYM	**C**		
				FN_{BMC}	-3.4%[(d)]	-3.8%[(d)]		
				TR_{BMC}	-3.7%[(d)]	-2.0% (NS)		
				TIB_{TrBMD}	-0.6%[(d)]	-1.0% (NS)		
				TIB_{CoBMD}	-2.0%[(d)]	-2.0% (NS)		
Foley et al., 2010 (6) (10 Jahre)	♂ und ♀ N: 124	POST 62.7±7.3	*Pedometer* Schrittzahl (Quartile)	*DEXA*	**%-Differenz für Schritt$_{Q4}$ (vs. Schritt$_{Q1}$)**			- mit höheren Alter erhöht sich der Effekt von KA - k.A. von Signifkanzen für die %-Differenz zwischen den I-Gruppen
					♀	♂		
				HIP_{BMD} (65 J)	+3.9%	+3.9%		
				HIP_{BMD} (75 J)	+7.4%	+5.4%		
				LS_{BMD} (65 J)	+5.1%	k.A. (NS)		
				LS_{BMD} (75 J)	+4.2%	k.A. (NS)		
Puntila et al., 2001 (80) (8-9 Jahre)	♀ N: 1873	POST 62.7±7.3	*FB* KA, WBA	*DEXA*	**Reduzierter jährlicher Knochen-schwund (%) für KA und WBA (je vs. C)**			
					KA	**WBA**		
				LS_{BMC}	27%, *p=.036*	60%, *p=.022*		
				LS_{BMD}	23%, *NS*	52%, *p=.029*		

C: Kontrollgruppe; **FB**: Fragebogen; **GYM**: Gymnastik; **J:** Jahre (Alter); **k.A.**: Keine Angabe; **KA**: Körperliche Aktivität; **KF**: Knochenfestigkeit; **NS**: Nicht signifikant; **PRÄ**: vor Eintritt der Menopause; **POST**: während / nach Eintritt der Menopause; **Q_1-Q_4**: Quartile; **T0**: Basiserhebung; **T1**: 1. Follow-up; **WBA**: *Weight-Bearing Activities*

[(a)] Abkürzungsverzeichnis siehe Tabelle 1

[(b)] Altersspanne ermittelt aus den gemittelten Altern pro Gruppe[)]

[(c)] prozentuale Effekte ermittelt aus den gemittelten Indikatoren der Knochenfestigkeit

[(d)] signifikant im 95%-Konfidenzintervall

Tabelle 5: Interventionsstudien (RCT, CT) im Erwachsenenalter

Studie	Studien-umfang	Altersspanne (Jahre)	Methode/Art KA/ Sport	Methode/ KF-Indikatoren [(a)]	Interventionseffekte	Bemerkung
Winters und Snow, 2000 (76)	♀ N: 51	PRÄ 30-45	12 Monate, 3x/W / RES- und Sprungtraining	*DEXA* FN_{BMD} TR_{BMD} TB_{BMD}	**%-Veränderung T0-T1 für I vs. C** +1.2%, *p<.05* vs. -0.3%, *NS* +2.7%, *p<.05* vs. +0.8%, *p<.05* +1.0%, *p<.05* vs. +0.2%, *NS*	- kein randomisiertes Studiendesign - %-Veränderung 6 Monate nach Interventionsende: I vs. C: FN_{BMD} -2.0%, *p<.05* vs. -0.1%, *NS* LS_{BMD} -0.7%, *NS* vs. +1.0%, *p<.05*
Winters-Stone und Snow, 2006 (73)	♀ N: 59	PRÄ 38.3±3.8 – 41.3±3.8 *(b)*	12 Monate, 3x/W / RES an Unter- (I_U) / Gesamtkörper (I_G)	*DEXA* TR_{BMD} LS_{BMD}	**%-Veränderung T0-T1 für I_U vs. I_G (vs. C)** +2.6% vs. +2.2% (vs. +0.7%), *p<.02* -0.3% vs. +1.3% (vs. -0.5%), *p=.06*	- kein randomisiertes Studiendesign - keine I-Effekte an TB_{BMD}, HIP_{BMD} und FN_{BMD}
Vainionpää et al., 2005 (72)	♀ N: 120	PRÄ 35-40	12 Monate, 3x/W / Sprung- und Laufprogramm	*DEXA, QUS* FN_{aBMD} IT_{aBMD} F^{total}_{aBMD} BUA	**%-Veränderung T0-T1 für I vs. C** +1.1% vs. -0,4%, *p=.003* +0.8% vs. -0,2% , *p=.029* +1.0% vs. -0,3%, *p=.006* +7.3% vs. +0,6%, *p=.015*	- keine I-Effekte an LS und Unterarmknochen
Vainionpää et al., 2007 (75)	♀ N: 80	PRÄ 35-40	12 Monate, 3x/W / Sprung- und Laufprogramm Einteilung in Subgruppen: I_{Q1} (<19 Übungen) bis I_{Q4} (>66 Übungen)	*DEXA, QCT* F^{mid}_{Cf} TIB^{prox}_{Cf} TIB^{prox}_{CoCSA} $TIB^{prox}_{CSMImax}$	**%-I-Effekt für I und C** +0.2%, *p=.033* **%-Differenz für I_{Q4} (vs. I_{Q1})** +1.2%, *p=.03* +0.5%, *p=.04* +2.5%, *p=.05*	- keine I-Effekte auf Indikatoren der KF am Oberschenkelhals
Singh et al., 2009 (78)	♀ N: 58	PRÄ 30-50	9 Monate, 2x/W / RES	*DEXA*	Keine sig. I-Effekte an TB_{BMD}, LS_{BMD}, HIP_{BMD}, ARM_{BMD} und LEG_{BMD}	
Vanni et al., 2010 (79)	♀ N: 27	PRÄ 39.6±0.4	28 Wochen, 2x/W / RES	*DEXA*	Keine sig. I-Effekte an FN_{BMD}, LS_{BMD}	
Helge et al., 2010 (8)	♀ N: 65	PRÄ 36.5±7.7	14 Wochen, ~2h/W / SOC (I_{SOC}), LAUF (I_{LAUF})	*DEXA, pQCT* TIB_{vBMD} TIB_{TrBMD}	**%-Veränderung für rechtes/linkes Bein** **I_{SOC}** **I_{LAUF}** +2.6%/+2.1%, *p<.01* 0.7%/1.1%, *p≤.05* +2.6%/+2.0%, *p≤.01* 1.4%/1.0%, *p≤.05*	- keine sig. Veränderungen in TB - keine sig. Veränderungen in C
Bemben et al., 2000 (77)	♀ N: 25	POST 41-60	6 Monate, 3x/W / RES (hohe (8x80% 1RM) vs. geringe Intensität (16x40% 1RM)	*DEXA*	Keine sig. I-Effekte an TB_{BMD}, LS_{BMD}, FN_{BMD}, TR_{BMD}, HIP_{BMD} und WT_{BMD}	- Tendenz zu verringerten BMDs nach der Intervention
Rhodes et al,. 2000 (83)	♀ N: 40	POST 65-75	6 Monate, 3x/W / RES (8x75% 1RM)	*DEXA*	Keine sig. I-Effekte an LS, FN, TR und WT	- ♀ mit Osteopenie
Maddalozzo und Snow, 2000 (71)	♂ und ♀ N: 54	POST 52.8±3.3 – 54.6±3.2 *(b)*	6 Monate, 3x/W / RES hohe (50-70% 1RM) vs. moderate Intensität (40-60% 1RM)	*DEXA* TR_{BMD} FN_{BMD} LS_{BMD}	**%-Veränderung T0-T1 für I_{hoch}** ♂ ♀ +1.3%, *p<.05* +2.0, *p<.05* -1.8%, *p<.05* +6.0%, *p<.05 (c)* +1.9%, *p<.05* --	- ♀ mit Östrogendefizit (keine Östrogenersatztherapie)
Bemben et al., 2011 (2)	♂ und ♀ N: 124	POST 55-74	40 Wochen, 2-3x/W / RES 4 verschiedene Intensitäten	*DEXA* LS_{BMD} TR_{BMD} TB_{BMD}	**%-Veränderung T0-T1 für alle Intensitäten:** ♂ ♀ +1.8%, *p=.054* +0.4, *p=.054* +0.5%- +1.5%, *p<.05* +0.5%- +1.5%, *p<.05* -0.6% (k.A. zu *p*) -0.5% (k.A. zu *p*)	- ♀ mit HRT - I-Effekt auch an HIP_{BMD} - Kein I-Effekt an Femur

Studie	Studien-umfang	Altersspanne (Jahre)	Methode/Art KA/ Sport	Methode/ KF-Indikatoren [(a)]	Interventionseffekte	Bemerkung
Kemmler et al., 2003 (84)	♀ N: 100	POST I: 55.1±3.4 C: 55.9±3.1	14 Monate, 4x/W / Kombinationstraining (Lauf-, Kraft- und Sprungtraining)	*DEXA, QCT* LS_{BMD} FN_{BMD} HIP_{BMD}	**%-Veränderung T0-T1 für I vs. C:** +1.3% vs. -1.2%, *p<.001 (für I und C)* -0.8%, *p<.05* vs. -1.8%, *p<.001* -0.3% (*NS)* vs. -0.8%, *p<.05*	- ♀ mit Osteopenie - kein randomisiertes Studiendesign - zusätzlich Kalziumsupplementation - Kein I-Effekt an ARM
Engelke et al., 2006 (70)	♀ N: 78	POST I: 55.1±3.3 C: 55.5±3.0	3 Jahre, 4x/W / Kombinationstraining (Lauf-, Kraft- und Sprungtraining)	*DEXA, QCT, QUS* LS_{BMD} LS_{TrBMD} LS_{CoBMD} HIP_{BMD} ARM^{dist} SOS BUA	**%-Veränderung T0-T1 für I vs. C:** +0.8%, *NS* vs. -3.3%, *p<.001* +1.1%, *NS* vs. -7.7%, *p<.001* +5.3%, *p<.001* vs. -2.6%, *p<.001* - 0.2% *NS* vs. -1.9%, *p<.001* - 2.8%, *p<.001* vs. -3.8%, *p<.001* +0.3% *NS* vs. -1.0%, *p<.001* - 0.3% *NS* vs. -5.4%, *p<.001*	- ♀ mit Osteopenie - kein randomisiertes Studiendesign - keine sig. Veränderungen im Femur
Bemben et al., 2010 (82)	♀ N: 55	POST 55-75	8 Monate, 3x/W / Kombinationstraining: RES (80% 1RM) + Körpervibration	*DEXA*	Keine sig. I-Effekte an TB_{BMD}, LS_{BMD}, FN_{BMD}, TR_{BMD}, HIP_{BMD} und RAD_{BMD}	- ♀ mit Östrogendefizit (ohne HRT) - kein randomisiertes Studiendesign
Korpelainen et al., 2006 (85)	♀ N: 160	POST I: 72.9±1.1 C: 72.8±1.2	30 Monate, 3x/W / Sprung- und Balancetraining	*DEXA, QUS* FN_{aBMD} TR_{aBMD} TR_{BMC} $RAD^{dist}{}_{aBMD}$ $RAD^{UD}{}_{aBMD}$	**%-Veränderung T0-T1 für I vs. C:** -0.6%, *NS* vs. -1.1%[(d)] -0.3%, *NS* vs. -1.6%[(d)] -2.9%[(d)] vs. -7.7%[(d)] -3.8%, *p=.001* vs. -3.1%, *p=.001* -3.1%, *p=.003* vs. -3.4%, *p=.01*	- keine sig. Veränderung für BUA und SOS
Borer et al., 2007 (69)	♀ N: 49	POST 50-65	15 Wochen, 5x/W / 4.8km Schnelles Gehen Intensität hoch: 88% VO_2max Intensität niedrig: 62% VO_2max	*DEXA* TB_{aBMD} LEG_{aBMD}	**%-Veränderung T0-T1 für $I_{88\%}$ vs. $I_{62\%}$:** +0.4% vs. -1.3% (k.A. zu *p*) +0.1% vs. -1.1% (k.A. zu *p*)	- HRT Subgruppe: kein Effekt von HRT auf die Veränderung der Indikatoren - An HIP_{aBMD}: sig. Zunahme in $I_{88\%}$ und sig. Abnahme in $I_{62\%}$
Karinkanta et al., 2007 (87)	♀ N: 149	POST 70-78	12 Wochen, 3x/W / - RES (I_{RES}) - Sprung-/Balancetraining (I_{BAL}) - Kombination RES und BAL (I_{COMB})	*pQCT* FN_Z	**%-Differenz für I_{RES} (vs. I_{COMB})** +5% (*NS*)	- Keine sig. Veränderungen an FN_{BMC} und $TIB^{sh}{}_{BSI}$ - COMB: 2% geringerer Knochenschwund im Vergleich zu C
Whiteford et al., 2010 (17)	♀ N: 143	POST 55-80	12 Monate, 3x/W / RES	*DEXA* TR_{BMD} HIP_{BMD}	**%-Veränderung T0-T1 für $I_{88\%}$ vs. $I_{62\%}$:** +2.2% vs. +2.2%, *p<0.5 für beide* +0.9% vs. +0.9%, *p<0.5 für beide*	- Keine sig. Veränderungen an TB_{BMD} und LS_{BMD}

C: Kontrollgruppe; **COMB**: Kombination; **I**: Intervention; **HRT**: Hormon-Ersatz-Therapie; **k.A.**: keine Angabe; **KF**: Knochenfestigkeit; **LAUF**: Ausdauerlaufen; **NS**: nicht signifikant; **PRÄ**: vor Eintritt der Menopause; **POST**: während / nach Eintritt der Menopause; **RES**: Resistenztraining; **RM**: Maximale Wiederholung (*Repetition Maximum*); **sig.**: signifikant; **SOC**: Fußball; **T0**: Basiserhebung; **T1:** 1. Follow-up; **x/W**: Anzahl pro Woche

[(a)] Abkürzungsverzeichnis siehe Tabelle 1

[(b)] Altersspanne ermittelt aus den gemittelten Altern pro Gruppe

[(c)] prozentuale Effekte ermittelt aus den gemittelten Indikatoren der Knochenfestigkeit

[(d)] signifikant im 95%-Konfidenzintervall

Appendix B

Impact of Physical Activity, Sedentary Behaviour and Muscular Strength on Bone Stiffness in 2-10-year-old Children. Cross-Sectional Results from the IDEFICS Study

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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

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Abstract

Background

Physical activity (PA), weight-bearing exercises (WBE) and muscle strength contribute to skeletal development, while sedentary behaviour (SB) adversely affects bone health. Previous studies examined the isolated effect of PA, SB or muscle strength on bone health, which was usually assessed by x-ray methods, in children. Little is known about the combined effects of these factors on bone stiffness (SI) assessed by quantitative ultrasound. We investigated the joint association of PA, SB and muscle strength on SI in children.

Methods

In 1,539 preschool (2-5 years) and 2,982 school children (6-10 years), data on calcaneal SI and on accelerometer-based sedentary time (SED), light (LPA), moderate (MPA) and vigorous PA (VPA) were available. Parents reported sports (WBE versus no WBE), leisure time PA and screen time of their children. Jumping distance and handgrip strength served as indicators for muscle strength. The association of PA, SB and muscle strength with SI was estimated by multivariate linear regression, stratified by age group. Models were adjusted for age, sex, country, fat-free mass, daylight duration, consumption of dairy products and PA, or respectively SB.

Results

Mean SI was similar in preschool (79.7±15.1) and school children (81.2±12.1). In both age groups, an additional 10 min/day in MPA or VPA increased the SI on average by 1% or 2%, respectively (p<.001). The negative association of SED with SI decreased after controlling for MVPA. LPA was not associated with SI. Furthermore, participation in WBE led to a 3% and 2% higher SI in preschool (p=0.005) and school children (p<.001), respectively. Although muscle strength significantly contributed to SI, it did not affect the associations of PA with SI. In contrast to objectively assessed PA, reported leisure time PA and screen time showed no remarkable association with SI.

Conclusion

This study suggests that already 10 min/day of additional VPA or the participation in WBE may be sufficient for a relevant increase in SI in children, taking muscle strength and SB into account. Our results support the importance of assessing accelerometer-based PA in large-scale studies. This may be important when deriving dose-response relationships between PA and bone health in children.

Keywords: Bone stiffness, physical activity, sedentary behaviour, accelerometer, quantitative ultrasound, quantitative evidence, weight-bearing exercise, muscle strength

Background

High levels of physical activity (PA) have been found to optimize skeletal development early in life, thus preventing age-related bone loss and osteoporotic fractures [1-4]. The positive impact of moderate (MPA), vigorous (VPA) or moderate-to-vigorous PA (MVPA) on bone health in children has been demonstrated in several observational studies [5-11]. In school-based interventions an osteogenic effect of WBE such as jumping or ballgames has been observed. The effect of high-impact PA has been largely explained by the muscle force and strength acting on bone [2, 12-18]. Thus, muscle strength and muscle mass play an important role in bone development during growth [19].

International PA guidelines for children from the World Health Organization (WHO) recommend one hour of MVPA per day, including VPA or bone-strengthening exercises on at least three days a week [20]. However, a large number of studies have indicated that most children spend insufficient time in MVPA [6, 7, 21-23]. The time of MVPA may be replaced by the increasing time children spend in sedentary behaviours such as watching television or playing computer games that may adversely affect bone health [5, 24, 25]. According to previous studies, the adverse effect of sedentary behaviours on bone health may be counteracted by additional high-impact PA [26, 27].

The variety of methods for assessing and operationalizing PA and bone health hamper the comparison of studies, particularly for investigating consistent dose-response relationships and bone-related PA recommendations. This is further complicated by the fact that usually only the isolated osteogenic effect of either habitual PA, different types of WBE or sedentary behaviours has been examined. The osteogenic effect of different PA intensities combined with sedentary behaviour in children is poorly investigated. In particular, there is a lack of quantitative evidence on the association of PA and WBE with bone health in children younger than five years [6].

In the IDEFICS study (Identification and prevention of dietary- and lifestyle- induced health effects in children and infants), a large European sample of children aged 2-10, bone stiffness index (SI), as an indicator for bone health, was measured using quantitative ultrasound (QUS) [28]. We comprehensively assessed habitual PA levels, sedentary behaviour and physical fitness, which made it possible for us to simultaneously investigate the association of these lifestyle factors with SI in children. In detail, we examined the effect of objectively measured average PA levels, SED, LPA, MPVA, VPA and MVPA as well as of reported leisure time PA, WBE and screen time on SI in preschool (2-<6 years) and school children (6-10 years).

We additionally investigated the association of muscular fitness and fat-free mass (FFM) on SI separately as well as in combination with PA and sedentary behaviour. Both, muscular fitness and FFM have been used as indicators for muscle strength and muscle mass in previous studies [8, 29-31].

Methods

Study sample

The IDEFICS study, a prospective population-based cohort study, examined more than 18,000 2-11-year-old children, from eight European countries (Sweden, Germany, Hungary, Italy, Cyprus, Spain, Belgium and Estonia) to investigate associations of biological and behavioural factors on lifestyle diseases. The study was conducted according to the standards of the Declaration of Helsinki. All participating centres obtained ethical approval from their responsible authority. Participating children and their parents provided oral and written informed consent for each examination and for the storage of personal data. Children and their parents were allowed to opt out of individual examination modules, e.g. blood collection, accelerometry or QUS measurements. The study design and examinations done have been described in detail elsewhere [32, 33]. Our sample is based on 16,228 children examined at baseline (T0, 2007/08), and 2,555 newly recruited children examined at follow-up (T1, 2009/10).

The exclusion criteria and the number of children included in this analysis are summarized in Figure 1. We considered children 2-10 years of age with their first QUS measurement at T0 (N=7,539) or T1 (N=3,842). We included children with valid QUS measurements and with no indication of impaired bone health, i.e. without diseases or medical treatments affecting the bone. Further inclusion criteria were, the availability of accelerometer measurements, parental-reported WBE, leisure time PA and screen time as well as data on FFM, daylight duration and the reported consumption of milk and dairy products (MDP). In school children, we considered only children who participated in fitness tests including jumping distance and handgrip strength. These restrictions left 1,539 preschool and 2,982 school children for analysis.

Bone stiffness

SI was measured on the left and right calcaneus using QUS (Achilles Lunar Insight^{TM} GE Healthcare, Milwaukee, WI, USA) and was based on two parameters, broadband ultrasound

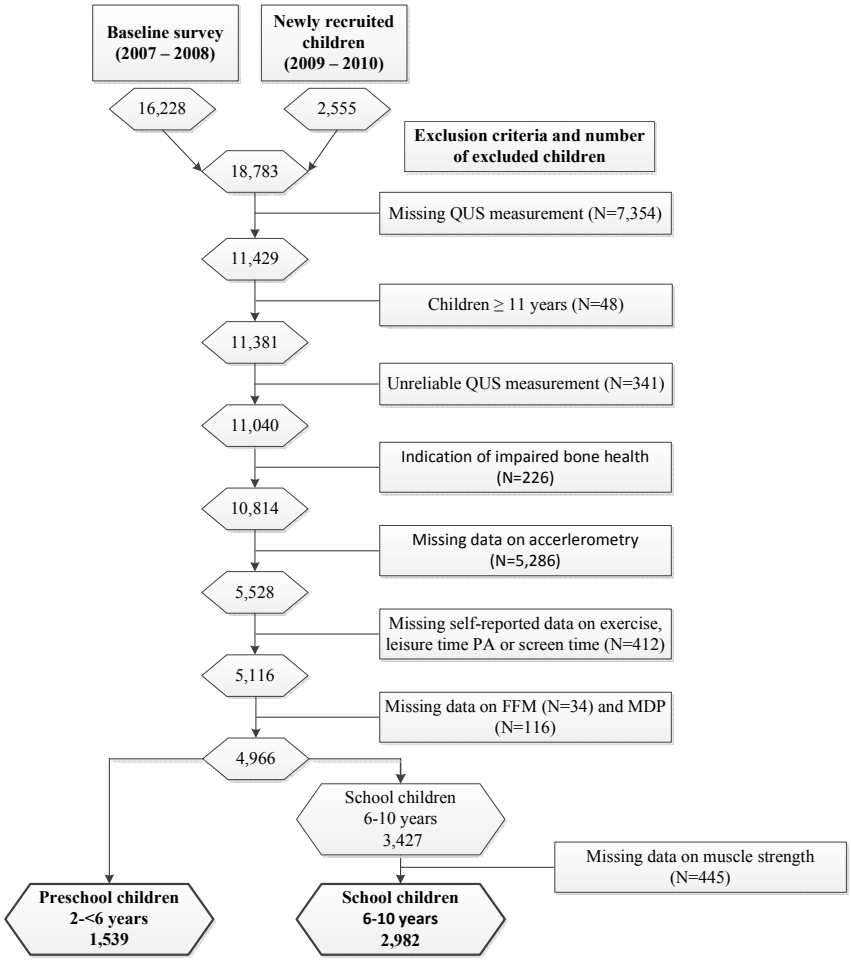

Figure 1
Number of included and excluded children per exclusion criteria for the analysis group

attenuation (dB/MHz) and speed of sound (m/sec) [34]. QUS measurements are correlated with DXA measurements and are used as a valid tool for indicating the risk of osteoporotic fractures [35, 36]. In children, however, the clinical usefulness of QUS has not yet been investigated, and comparison studies showed inconsistent correlations with DXA [34, 37]. The detailed method and application of QUS is described elsewhere [28, 38, 39]. To examine the reliability of Achilles devices, 60 children were repeatedly measured on either the right (N=30) or left (N=30) calcaneus. The precision of Achilles measurements was calculated using the percent root-mean-square coefficient of variation (CV_{RMS}) in accordance with the Conference of Radiation Control Program Directors (CRCPD) Task Force on Bone Densitometry [40]. CV_{RMS} for SI was 9.2% on the right foot and 7.2% on the left foot. Furthermore, in our sample, the absolute difference of the left and right SI measurement was on average 10.3 units (standard deviation 12.5 units) and ranged from 0 to 112 units. A small reliability study that was conducted in a convenience sample (N=91) based on five different Achilles devices used in the IDEFICS study confirmed the significant discrepancy of the SI measurements between the left and right foot (multilevel regression analysis: β=0.45, p=0.05,

unpublished data). To control for this discrepancy we set a limit for the absolute SI difference between SI of the left and right calcaneus and excluded 3% of the QUS sample having the highest SI difference (97th SI percentile: 45 units SI difference). We calculated the mean SI of both feet as a proxy for bone status of the lower limbs and hip.

Physical activity and sedentary behaviour

PA was objectively measured using Actigraph uniaxial accelerometers (ActiTrainer or GT1M; Actigraph, LLC, Pensacola, FL, USA). Children had to wear the device on their right hip and had to take it off during water-based activities and bedtime. Data were considered valid when the child wore the accelerometer for three consecutive days, including one weekend day, for at least six hours per day. In our analysis, we used accelerometer data with 60 second (s) epoch. Non-wearing time was defined as 20 minutes or more of consecutive zero counts [21]. The average PA levels of the children were defined by counts per minute (cpm). Intensity levels were classified as sedentary time (SED, ≤100 cpm), LPA (>100-<2296 cpm), MPA (≥2296-<4012 cpm), VPA (≥4012 cpm) and MVPA (≥2296 cpm) based on the cut-off values proposed by Evenson [41]. For each intensity level the cumulative duration was calculated in minutes per day. The total valid wearing time of the device was assessed and expressed as average hours per day.

Children's WBE, leisure time PA and screen time were reported by parents using a questionnaire. The variable WBE was based on the question "What kind of sport does your child do in a sports club?" Four types of sport typical for each respective country and an open category to record sports that were not listed were offered as response possibilities. All types of sport were classified according to their loading and categorised into: (i) moderate or high mechanical loads on the lower limbs (ballgames, gymnastics, dancing, skating, martial arts, and athletics), and (ii) no or low mechanical loads (swimming, biking and horseback riding) or no sports. The latter also included children for whom no information on sports was available.

Based on the "Outdoor Playtime Recall Questions" [42], parents were asked how many hours (h) and minutes (m) their child spent playing outdoors on a typical weekday (weekd_h, weekd_m) and weekend day (weeken_h, weeken_m) the previous month. In addition, parents were asked how many hours (club_h) and minutes (club_m) per week the child spent doing sport in a sports club. The variable leisure time PA was calculated as 5*(weekd_h + weekd_m/60) + 2*(weeken_h + weeken_m/60) + (club_h + club_min/60) and expressed as hours per week.

Sedentary behaviours, such as watching TV or playing computer games were used as a proxy for reported sedentary time [27]. Parents were asked to recall the usual duration their child watched (i) TV, videos, and DVDs, and (ii) the duration their child used the computer and 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, <30min =1, <1h =2, 1-<2h =3, 2-3h =4, and >3h =5. Screen time was calculated separately for weekdays (weekd_score) and weekend days (weeken_score) by adding up the converted responses of questions (i) and (ii). The total screen time in hours per week was calculated as [(weekd_score*5) + (weeken_score*2)].

Assessment of muscle strength

Physical fitness tests were conducted only among school children and adapted from the ALPHA (Assessing Levels of Physical Activity) and FITNESSGRAM test battery [29, 43, 44]. Jumping distance and handgrip strength were considered to indicate muscle strength in the lower and upper limbs, respectively [29]. Jumping distance was assessed using a standing broad jump test, measured to the nearest 1.0 cm. Handgrip strength was measured to the nearest 0.1 kg using a digital handgrip dynamometer (Takei TKK 5401/5101). Each child had two attempts per test and the maximum value of both attempts was considered for our analysis.

Assessment of fat-free mass

FFM (kg) was used as a proxy for skeletal muscle mass, which has been reported to be positively associated with bone strength [30, 31]. It was calculated based on height (Stadiometer SECA 225), weight, and leg-to-leg bioelectrical impedance (both measured with Tanita scale BC420 MA) using the Tyrrell formula [45]. As height and weight are strongly correlated with FFM, we did not consider either of them as an adjustment variable. In the current analysis, on the one hand, the association of FFM with SI was examined without considering PA behaviour. On the other hand, the association between PA behaviour and SI was additionally controlled for FFM, which is in accordance with previous studies [7-9].

Assessment of co-variables

MDP were considered as an indicator for calcium intake and estimated based on the habitual consumption frequency of milk, yoghurt, and cheese as reported by parents using a food frequency questionnaire that was developed in the IDEFICS study [46, 47]. The response categories ranged from ‘Never/less than once a week’ to ‘Four or more times per day’ and were converted into the weekly frequency of MDP consumption.

Exposure to sunlight is the most important source for vitamin D synthesis that contributes to bone mineralization [48]. Hence, we adjusted for mean daylight duration (±0.1 hours) which was calculated for each examination month in each location, using astronomical tables [49].

Statistical analyses

The associations of PA, sedentary behaviour and muscle strength with SI were analysed using multivariate linear regression models. Data were checked for normality and linearity using residual plots. Regression analyses were conducted for each variable of PA behaviour (LPA, MPA VPA, MVPA, average PA level, WBE, leisure time PA), sedentary behaviour (SED, screen time) and muscle strength (FFM, jumping distance, handgrip strength). Objectively measured and reported variables were analysed and presented separately. In a first model, we adjusted for age, sex and country (Model 1). The second model was additionally adjusted for FFM (unless FFM was an independent variable), MDP and daylight duration (Model 2). A third model was conducted to additionally adjust model 2 for the time of either accelerometer-based SED or MVPA, or respective reported PA/ sedentary behaviours (Model 3). Thus, except for the average PA level, each independent variable was additionally adjusted as follows: the *PA intensities LPA, MPA, VPA and MVPA* were each adjusted for SED; *SED* was adjusted for MVPA; *variables for muscle strength* for average PA level; *screen time* for reported leisure time PA; and *leisure time PA and WBE* for reported screen time. In school children, we additionally adjusted model 3 for jumping distance and handgrip strength (Model 4). Except for the model for average PA level, all models that included accelerometer data were adjusted for valid wearing time of the accelerometer. To allow better interpretation of the regression coefficients, accelerometer-based variables were converted as follows: average PA level as 100 cpm, SED and LPA as hours/day and MPA, VPA and MVPA as 10 min/day.

Analyses were conducted for boys and girls together, since no moderating effect of sex on the association between PA and SI was detected. Regression models were stratified for preschool (2-<6 years) and school children (6-10 years). This was decided due to the lack of evidence in children younger than six years, and the lack of fitness data in preschool children. Furthermore, previously published age-, sex- and height-specific SI percentile values indicated a decrease of SI in preschool children and an increase in school children [28].

Based on the described models, we performed sensitivity analyses with accelerometer data using 15s epochs that were not available for all children (N=3,519).

Level of significance was set at α=0.05. Analyses were performed using SAS 9.3 (SAS Institute Inc., Cary, NC, USA).

Results

Study sample characteristics

The characteristics of the study sample are shown in Table 1. Differences in mean SI by age and sex were negligible. Preschool and school children wore the accelerometer on average 11.5 and 12.2 hours per day, respectively. The accelerometer-based average PA levels were slightly higher in preschool compared to school children. The mean time spent in LPA, MPA and VPA as well as the reported leisure time PA were similar for both age groups. School children were more engaged in WBE, but also reached a higher mean of SED and reported screen time compared to preschool children. In both age groups, boys had higher mean values in all PA variables, except LPA, as well as slightly higher SED and reported screen time. Finally, compared to school girls, school boys had slightly higher mean values in the fitness tests.

Tables 2 and 3 present the results of models 1-4 for objectively and subjectively assessed variables, respectively. The explained variance of SI by PA, sedentary behaviour and muscle strength was about 18%-20% in preschool and 24%-27% in school children, which was consistent throughout the models. Regression coefficients were not or only slightly reduced by adjustment for FFM, MDP and daylight duration (model 2), PA, or respectively sedentary behaviour (model 3) and muscle strength (model 4).

PA behaviour and SI

We observed a positive association of accelerometer-based PA levels with SI in preschool (β=0.95, $p<.001$) and in school children (β=1.02, $p<.001$). When classified into PA intensities and based on model 3, our results suggest that a 10 minute increase in MPA per day would lead to an about 0.8 unit higher SI in both age groups ($p\leq.001$). With the same increase in VPA, the association with SI was about 1.5-2-fold as high as the association between MPA and SI in preschool (β=1.15, $p=.006$) and school children (β=1.59, $p<.001$). LPA showed no clear association with SI in both age groups (see Table 2).

Considering reported PA, we observed higher SI values of about 2.5 units for preschool ($p=0.005$) and 1.7 units for school children ($p<.001$) who participated in WBE compared to those performing no WBE or no exercise at all. In contrast, for leisure time PA, we observed a 0.07 and 0.05 unit increase in SI for an additional hour per week in preschool ($p=0.05$) and school children ($p=0.015$), respectively (see Table 3).

Table 1 Characteristics of preschool (2-<6 years) and school (6-10 years) children, stratified by sex

	Preschool children (2-<6 years)			Primary school children (6-10 years)		
	Boys	**Girls**	**All**	**Boys**	**Girls**	**All**
	mean±SD or N (%)	**mean±SD or N (%)**	**mean±SD or N (%)**	**mean±SD or N (%)**	**mean±SD or N (%)**	**mean±SD or N (%)**
Number	814	725	1,539	1,419	1,563	2,982
Age (years)	4.4±0.9	4.5±0.9	4.5±0.9	8.1±1.2	8.1±1.2	8.1±1.2
Anthropometric measures						
Bone stiffness index	79.3±14.7	80.1±15.6	79.7±15.1	82.0±12.5	80.5±11.6	81.2±12.1
Body height (cm)	115.9±9.8	114.6±9.4	115.3±9.6	135.2±8.9	134.7±9.1	134.9±9.0
Body weight (kg)	21.8±5.4	21.1±4.9	21.4±5.2	32.5±8.7	32.1±8.3	32.3±8.5
Fat-free mass (kg)	15.7±3.6	13.9±3.3	14.9±3.6	23.1±4.0	21.3±4.0	22.2±4.1
Objectively measured PA and sedentary behaviours						
Sedentary time (hours/day)	4.4±1.5	4.5±1.4	4.4±1.5	5.6±1.6	5.7±1.5	5.7±1.5
Light PA (hours/day)	6.5±1.0	6.4±1.0	6.4±1.0	5.8±1.1	5.8±1.1	5.8±1.1
Moderate PA (min/day)	38±18	29±14	34±17	39±19	29±13	34±17
Vigorous PA (min/day)	7±7	6±5	6±6	9±8	7±6	8±7
Moderate-to-vigorous PA (min/day)	45±23	35±18	40±21	48±25	35±18	42±22
Moderate-to-vigorous PA ≥ 60 min/day (WHO, 2011)	202 (24.8)	75 (10.3)	277 (18.0)	433 (30.5)	162 (10.4)	595 (20.0)
PA levels (counts/ minute)	653±173	586±147	622±165	578±169	507±138	541±158
Wearing time (average hours/day)	11.6±1.7	11.5±1.7	11.5±1.7	12.2±1.7	12.1±1.7	12.2±1.7
Reported PA and sedentary behaviours						
High-to-moderate WBE	182 (22.4)	222 (30.6)	404 (26.3)	822 (57.9)	817 (52.3)	1,639 (55.0)
No-impact WBE / no exercise	632 (77.6)	503 (69.4)	1,135 (73.8)	597 (42.1)	746 (47.7)	1,343 (45.0)
Leisure time PA (hours/week)	17.8±10.1	17.5±9.8	17.7±9.9	18.9±9.8	17.2±9.8	18.0±9.8
Screen time (hours/week)	12.1±7.0	10.6±6.4	11.4±6.7	15.3±8.1	13.3±7.3	14.3±7.7
Measures of muscle strength						
Jumping distance (cm)				121±25	114±24	117±25
Handgrip strength (kg)				14.4±3.9	13.2±3.4	13.8±3.7
Potential confounding lifestyle factors						
Dairy products (frequency/week)	22±12	21±11	21±12	20±12	19±12	20±12
Daylight duration (hours/week)	11.0±2.8	10.8±2.6	10.9±2.7	10.8±2.6	10.7±2.6	10.7±2.6

PA – physical activity; WBE – weight-bearing exercises; SD – standard deviation

Table 2 Multivariate linear regression investigating the association of accelerometer-based PA data with SI, by age group

	Model 1			Model 2			Model 3*			Model 4		
	Adjusted for age, sex, country			Model 1 + adjusted for FFM, MDP, daylight duration			Model 2 + adjusted for either PA or/and sedentary time			Model 3 + adjusted for muscle strength		
	β	*p* value	R^2 (%)	β	*p* value	R^2 (%)	β	*p* value	R^2 (%)	β	*p* value	R^2 (%)
Preschool children (N=1,539)												
Sedentary time (hour/day)	-0.88	0.006	18.3	-0.74	0.022	19.1	-0.40	0.24	19.5			
Light PA (hour/day)	0.19	0.62	17.9	0.01	0.97	18.8	-0.39	0.33	19.1			
Moderate PA (per 10 min/day)	0.79	<.001	18.5	0.85	<.001	19.5	0.76	0.002	19.6			
Vigorous PA (per 10 min/day)	1.19	0.05	18.1	1.32	0.033	19.0	1.15	0.06	19.2			
MVPA (per 10 min/day)	0.61	<.001	18.5	0.66	<.001	19.5	0.59	0.002	19.5			
PA levels (per 100 cpm)	1.00	<.001	18.7	0.95	<.001	19.5						
FFM (kg)[#]	-0.42	<.001	18.3	-0.41	<.001	18.6	-0.41	<.001	19.5			
School children (N=2,982)												
Sedentary time (hour/day)	-0.61	<.001	23.9	-0.78	<.001	25.8	-0.43	0.011	26.8	-0.46	0.007	27.0
Light PA (hour/day)	0.27	0.17	23.6	0.44	0.023	25.3	-0.0008	0.997	25.8	0.09	0.69	26.2
Moderate PA (per 10 min/day)	0.80	<.001	24.6	0.93	<.001	26.5	0.82	<.001	26.7	0.76	<.001	26.9
Vigorous PA (per 10 min/day)	1.61	<.001	24.4	1.74	<.001	26.1	1.59	<.001	26.5	1.42	<.001	26.8
MVPA (per 10 min/day)	0.64	<.001	24.7	0.73	<.001	26.6	0.65	<.001	26.8	0.60	<.001	27.0
PA level (per 100 cpm)	0.84	<.001	24.5	1.02	<.001	26.6				0.96	<.001	26.9
FFM (kg)	0.45	<.001	25.1	0.45	<.001	25.1	0.50	<.001	26.6	0.42	<.001	26.9
Jumping distance (10 cm)	0.42	<.001	24.0	0.36	<.001	25.5	0.28	0.002	26.8			
Handgrip strength (kg)[#]	0.42	<.001	24.7	0.19	0.013	25.3	0.18	0.022	26.7			

cpm - average counts per minute; FFM - fat-free-mass; MDP - milk and dairy products; MVPA - moderate-to-vigorous physical activity; PA - physical activity

*In Model 3, the *independent variables* were (in addition to Model 2) adjusted as follows: *sedentary time*: moderate and vigorous PA; *light PA, moderate PA, vigorous PA and MVPA:* sedentary time; *musculoskeletal fitness and FFM*: PA level; *PA level:* no additional adjustment for other accelerometer-based variables.

[#]In Model 2, FFM was adjusted for MDP and daylight duration.

Table 3 Multivariate linear regression investigating the association of reported PA data with SI, by age group

	Model 1			Model 2			Model 3*			Model 4		
	Adjusted for age, sex, country			Model 1 + adjusted for FFM, MDP, daylight duration			Model 2 + adjusted for either PA or SB			Model 3 + adjusted for muscle strength		
	β	*p* value	R^2	β	*p* value	R^2	β	*p* value	R^2	β	*p* value	R^2
Preschool children (N=1,539)												
Screen time (hours/week)	-0.07	0.22	17.8	-0.04	0.52	18.7	-0.04	0.52	18.9			
Leisure time PA (hours/week)	0.07	0.05	17.9	0.07	0.05	18.8	0.07	0.05	18.9			
WBE Impact WBE Ref: no exercise/WBE	2.11	0.015	18.0	2.46	0.005	19.1	2.45	0.005	19.1			
School children (N=2,982)												
Screen time (hours/week)	0.002	0.95	23.5	-0.01	0.62	25.1	-0.01	0.64	25.3	-0.006	0.83	25.7
Leisure time PA (hours/week)	0.05	0.020	23.6	0.05	0.015	25.3	0.05	0.015	25.3	0.04	0.029	25.7
WBE Impact WBE Ref: no exercise/WBE	1.75	<.001	24.0	1.68	<.001	25.6	1.67	<.001	25.6	1.50	<.001	25.9

PA – physical activity; WBE - weight-bearing exercise

*In Model 3, the *independent variables* were (in addition to Model 2) adjusted as follows: *screen time*: leisure time PA; *leisure time PA* and *WBE:* screen time.

Sedentary behaviour and SI

Accelerometer-based SED was negatively associated with SI in preschool and school children. After adjusting for MVPA (Model 3), the association between SED and SI was reduced by about 45% in preschool (β_{Model2}=-0.74, p=0.022 versus β_{Model3}=-0.40, p=0. 24) and school children (β_{Model2}=-0.78, p>.001 versus β_{Model3}=-0.43, p=0.011). No association was found between the reported screen time and SI.

Muscle strength and SI

In preschool children, FFM was negatively associated with SI (β=-0.41, p<.001). In school children on the other hand, FFM and muscle strength were positively associated with SI. In the latter, an additional kg of FFM corresponded to a 0.5 unit (p<.001) higher SI. Furthermore, every 10 cm increase in jumping distance and every 1 kg increase in handgrip strength resulted in a 0.3 (p=0.002) or 0.2 units (p=0.022) higher SI, respectively.

Discussion

This study provides quantitative evidence on the association of objectively measured PA and sedentary time as well as of muscle strength with SI based on a large sample of 2-10-year-old children.

Overall, high-impact PA such as VPA and WBE substantially contributed to a higher SI already as from preschool age, while no such effect was found for LPA. The observed association of high-impact PA with SI in preschool and school children was comparable. Our data indicate a decline of the osteogenic effect of PA with decreasing intensity. While 10 minutes of additional VPA per day lead to an almost 2% higher SI, the same increase in MPA showed a 1% higher SI. In addition, our results indicate, that MVPA partially reduces the adverse effect of SED on SI.

Previous observational studies support our findings that time spent in VPA appears to be more strongly associated with indicators of bone strength in children than time spent in LPA, MPA or MVPA [9, 50]. Furthermore, our results show similar associations as found in studies that examined the impact of MVPA and VPA on bone mineral content (BMC) or density (BMD). For instance, the Southampton Women's Survey, the only study found involving 4-year-old children, observed a 1.4% higher BMC for 10 minutes of additional MVPA per day [5]. The Iowa study reported that, in 5-year-old children, an extra 10 minutes in VPA per day lead to a 3% higher BMC. In 9-10-year-old children from the European Youth Heart Study, additional 10 minutes of VPA were associated with a 1-2% higher BMD [9]. According to Kriemler et al., a change of 1.8-2.8% in bone mass by PA or exercise intervention may be of relevance

[8]. Following Kriemler et al., our results suggest that at least an additional 10 minutes in VPA or 20 minutes in MPA per day would be necessary to achieve a relevant increase in SI. However, it should be kept in mind that SI is more an indicator for bone strength than bone mass [34]. Nevertheless, on the one hand, such an increase in SI would be more realistic and relevant for children who have not reached their optimum SI for age and sex [28]. On the other hand, the osteogenic effect in children who already perform high PA levels may reach a plateau after a certain time spent in these intensities. This would be an interesting issue to examine in a longitudinal perspective.

A stronger effect of MVPA or VPA on BMC in boys than in girls has been reported in previous studies [6-9]. For instance, comparing the highest tertile of VPA (72 min/day) to the lowest tertile (22 min/day), Kriemler et al. observed a 0.19 g difference of BMC in girls and a 1.22 g difference in boys. That is a 6-fold higher effect in boys than in girls [8]. Similarly, in the longitudinal perspective of the Iowa study, Janz et al. found that an additional 30 minutes of MVPA per day at age 5 lead to a 4.5% and 6.7% higher BMC in girls and boys at age 8, respectively. That is an almost 50% higher BMC accrual in boys compared to girls [6]. The researchers explained this sex-difference due to higher PA levels in boys [6, 8, 12]. Likewise, the boys in our study spent on average more time in MVPA than girls. Although we have found no moderating effect of sex on the association between PA and SI, we performed a sensitivity analysis stratified by sex, which was based on Model 3. The association of MVPA and VPA with SI was only slightly stronger in boys than in girls. For every additional 10 minutes in MVPA per day we observed a 60% higher increase SI in boys (β=0.48, p<.001) compared to girls (β=0.30, p=0.06). For the same increase in VPA, boys had only a 20% higher increase SI compared to girls (β_{boys}=1.23, p<.001 vs. β_{girls}=1.02, p=0.029). On the one hand, a 60% higher increase SI in boys is in line with the reported sex-difference from the Iowa study [6]. On the other hand, a 0.2 unit higher increase in SI for additional 10 minutes in MVPA, i.e. a 1.2 unit higher SI increase for an additional hour in MVPA appears to be a negligible sex-difference. However, some researchers have raised the point that boys might potentially have a higher genetically determined responsiveness of bones to PA and exercise compared to girls. Until today there is no evidence of this in children [8].

Contrary to objectively measured PA, reported leisure time PA was only weakly associated with SI in our study. School children whose parents reported 4 hours more in leisure time PA per week, i.e. approximately 30 minutes more leisure time PA per day, only showed a 0.3-0.4% higher SI. The weaker association between reported PA and bone indices compared to objectively assessed PA is consistent with findings from the Iowa study [51]. However, we

observed a strong positive association between subjectively assessed WBE and SI. Preschool and school children whose parents reported participation in WBE had a 3.1% and 2.1% higher SI, respectively compared to children that did not participate in WBE or exercise at all. We conclude that both, objectively measured PA as well as reported WBE, appear to be valuable indicators to investigate dose-response relationships of the impact of PA on bone.

The osteogenic effect of high-impact PA such as WBE has been proven in many school-based intervention programs involving jumping exercises. A 3-8% higher BMC was observed in children who participated in such programs lasting 7-9 months for at least three times per week compared to their peers who did not [13-15, 17]. Taking into account the reported periods of those school-based intervention programs, a dose-response effect of WBE on bone accrual can be suggested [2, 12-14, 18]. This suggestion is partly in line with the current WHO guidelines that recommend bone-strengthening exercises on at least three days a week [20]. However, there is a lack of evidence regarding the optimal dose of habitual PA for an adequate skeletal development in children. Our results indicate a positive association of MPA and especially of VPA with SI, although the prevalence of children who spent the recommended 60 minutes per day in MVPA was only about 20%. In a case-control study, embedded in the IDEFICS project, we have observed that children who spent less than 30 minutes in MVPA per day, i.e. less than 4.2% of their total PA, did have a 70% increased risk for a low SI compared to children who spent more than 46 minutes per day, i.e. more than 6.7% of their total PA in MVPA [52]. The latter result suggests that 30 minutes MVPA per day is not sufficient for an optimal SI. However, the optimal dose of habitual PA as well as of specific WBE programmes and their sustainable effect on bone accrual needs to be further investigated in longitudinal studies including intervention programs [15].

Our data support the beneficial osteogenic effect of muscle strength in school children. Currently, there are heterogeneous findings regarding the mediating role of muscle strength on the association between PA and SI [1, 8, 16]. After controlling for muscle strength, we only observed a slightly reduced association of MPA and VPA with SI. Although muscular activity is known as the largest mechanical load that forces the bone to adapt [19] high impact PA and muscle strength may be independently important for skeletal development. Considering FFM as an indicator for skeletal muscle mass, our results confirm a positive association with SI only in school children [8]. In preschool age, FFM was negatively associated with SI. This may be explained by the contribution of the higher proportion of organ tissues and the reduced proportion of skeletal muscle mass to FFM in early life [30, 53]. Furthermore, we calculated FFM accounting for height, a variable that was recently reported

to be negatively associated with SI in preschool age [28]. This finding indicates that FFM is not a suitable indicator for skeletal muscle mass in preschool children.

The application of QUS in large-scale studies in children is scarce. The few studies that used QUS mostly applied different devices, assessed PA using questionnaires or examined adolescents [25, 54-56]. This limits comparability with our findings. Only the ChiBS study applied the same QUS device as the one used in our study and assessed PA intensities by accelerometry, using the same cut-offs, but set at 15s epochs. In this study, a negative association of SED and a positive association of VPA with SI among 6-12-year-old children was observed [25]. These results support our findings.

While the strength of this study lies in the large sample size, the wide age range of children, and the application of parent-reported and objectively assessed PA, the interpretation of our data must consider the cross-sectional design.

We are aware of the fact that the minimum wearing time of three consecutive days for at least six hours per day may result in an underestimation of the true time a child spent in PA [5, 8, 51]. Nevertheless, the average wearing time per day was 11-12 hours.

The advantage of assessing VPA in children by using 15s epochs has been reported previously [57]. Shorter epochs that vary below 15s may better collect short and sporadic activity bouts that correspond to the natural activity pattern of a child [7, 23]. However, our sensitivity analyses revealed 43% and 16% weaker associations between accelerometer-based PA and SI in preschool and school children, respectively, when using 15s epochs compared to 60s epochs, taking the smaller sample size into account.

Another limitation is the missing fitness data in preschool children that hindered us from comparing the impact of muscle strength on SI between preschool and school age.

Finally, we cannot be sure whether all children were pre-pubertal since no information on maturity stages was available. To rule out the positive effect of oestrogens on the bone's sensitivity to PA in older girls, we performed sensitivity analysis in girls younger than 9 years and observed similar associations between PA and SI [18].

Conclusion

Our findings suggest that accelerometer-based PA and QUS-based bone indices may contribute towards determining potential dose-response relationships in children. This should be taken into consideration when planning further large-scale studies. Our study highlights the importance of high-impact and intense PA rather than light PA for optimizing

SI, as a proxy for bone strength in 2-10-year-old children. The participation in WBE, or respectively 10-20 minutes of extra MPA and VPA per day appear to be sufficient for a relevant increase in SI.

Abbreviations

ALPHA, Assessing Levels of Physical Activity; BMC, Bone Mineral Content; BMD Bone Mineral Density; ChiBS, Children's Body composition and Stress; cpm, counts per minute; FFM, Fat-Free Mass; IDEFICS, Identification and prevention of Dietary- and lifestyle-induced health EFfects In Children and infantS; LPA, Light Physical Activity; MDP, Milk and Dairy Products; MPA, Moderate Physical Activity; MVPA, Moderate-to-Vigorous Physical Activity; PA, Physical Activity; s, seconds; SAS, Statistical Analysis Software; SB, Sedentary Behaviour; SED, Sedentary Time; SI, Bone Stiffness; VPA, Vigorous Physical Activity; WBE, Weight-Bearing Exercises; WHO, World Health Organization

Competing interests

The authors state that they have no conflicts of interest.

Authors' contributions

Authors' role: Study design: WA, SM, TV, DM, and YP. Study conduct and data collection: All authors. Data analysis, interpretation, and drafting manuscript: DH, CB, and WA. DH, CB, and WA take responsibility for the integrity of the data analysis. All authors read, revised, and approved the final manuscript.

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Appendix C

Association between Bone Stiffness and Nutritional Biomarkers combined with Weight-Bearing Exercise, Physical Activity, and Sedentary Time in Preadolescent Children. A Case-Control Study

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Association between bone stiffness and nutritional biomarkers combined with weight-bearing exercise, physical activity, and sedentary time in preadolescent children. A case-control study

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Abstract

Physical activity (PA) and micronutrients such as calcium (Ca), vitamin D (25OHD), and phosphate (PO) are important determinants of skeletal development. This case-control study examined the association of these nutritional biomarkers and different PA behaviours, such as habitual PA, weight-bearing exercise (WBE) and sedentary time (SED) with bone stiffness (SI) in 1819 2-9-year-old children from the IDEFICS study (2007-2008). SI was measured on the calcaneus using quantitative ultrasound. Serum and urine Ca and PO and serum 25OHD were determined. Children's sports activities were reported by parents using a standardized questionnaire. A subsample of 1089 children had accelerometer-based PA data (counts per minute, cpm). Moderate-to-vigorous PA (MVPA) and SED were estimated. Children with poor SI (below the 15th age-/sex-/height-specific percentile) were defined as cases (N=603). Randomly selected controls (N=1216) were matched by age, sex, and country. Odds ratios (OR) for poor SI were calculated by conditional logistic regression for all biomarkers and PA behaviour variables separately and combined (expressed as tertiles and dichotomised variables, respectively). ORs were adjusted for fat-free mass, dairy product consumption, and daylight duration. We observed increased ORs for no sports (OR=1.39, $p<0.05$), PA levels below 524 cpm (OR=1.85, $p<0.05$) and MVPA below 4.2% a day (OR=1.69, $p<0.05$) compared to WBE, high PA levels (<688 cpm) and high MVPA (6.7%), respectively. SED was not associated with SI. ORs were moderately elevated for low serum Ca and 25OHD. However, biomarkers were not statistically significantly associated with SI and did not modify the association between PA behaviours and SI. Although nutritional biomarkers appear to play a minor role compared to the osteogenic effect of PA and WBE, it is noteworthy that the highest risk for poor SI was observed for no sports or low MVPA combined with lower serum Ca (<2.5mmol/l) or lower 25OHD (<43.0 nmol/l).

Keywords: bone health, weight-bearing exercise, case-control study, epidemiology, quantitative ultrasound, bone stiffness

1. Introduction

Physical activity (PA) and micronutrients such as calcium and vitamin D are important modifiable determinants of bone mineralization during growth that may optimize peak bone mass and reduce the risk of osteoporotic fractures in later life [1-4].

In particular, high-impact PA such as weight-bearing exercise (WBE) or moderate-to-vigorous PA (MVPA) improve bone strength in early life and appear to counteract the adverse effect of sedentary time (SED) on bone health [1, 5-10]. An even higher beneficial effect of high-impact PA in combination with increased calcium intake or supplementation has been observed [11-15], while habitual calcium intake alone has shown weak or no osteogenic effects [16].

The modest osteogenic effect of calcium intake can be explained by the complex homeostasis of calcium and the fact that only 10-30% of the calcium intake is absorbed in the intestines, enters the blood circulation, and is thus available for bone mineralization. In other words, calcium intake influences serum calcium (sCa) levels although the amount does not equal the circulating calcium, which is the key determinant of bone mineralization [17-21]. However, the majority of epidemiological studies in children focused more on the effects of calcium intake than that of circulating calcium [6, 11, 13-16].

Bone mineralization is dependent on the calcium homeostasis, which is regulated by the intestinal- and renal-dependent calcium transport, where serum 25-hydroxyvitamin D (25OHD) and phosphate (sPO) act as modifying factors [17, 18]. In brief, 25OHD or respectively its biological active metabolite calcitriol [$1,25(OH)_2D$] is responsible for the calcium absorption in the intestine. Thus, vitamin D deficiency, i.e. low 25OHD levels, decreases calcium absorption and leads to insufficient sCa [2]. Serum PO appears in bone as calcium-phosphate hydroxyapatite, which is required for bone mineralization. However, an excess phosphate intake results in high sPO levels that decrease the synthesis of $1,25(OH)_2D$ and thus reduce calcium absorption and sCa levels [18, 19]. Low sCa levels indicate an impaired calcium homeostasis. To maintain calcium homeostasis, low sCa levels stimulate the secretion of parathyroid hormones (PTH), which mobilise osteoclasts to release calcium from the skeleton into the blood circulation. This calcium removal from bone impairs bone mineralisation or leads to bone loss [17, 18, 22].

The complex mechanism of sCa, sPO and 25OHD has mostly been investigated in children with bone-related diseases such as chronic kidney disease, rickets or cystic fibrosis, which are associated with increased urine calcium (uCa) and phosphate (uPO) [2, 22-31]. These studies

mostly focused on vitamin D deficiency as a determinant of impaired bone health and only partly examined sCa and sPO. In particular, the understanding of sPO metabolism lags behind that of the metabolism of sCa and 25OHD.

There is insufficient evidence on how these biomarkers of the calcium-bone-homeostasis are associated with bone status in apparently healthy children, particularly in light of the human lifestyle nowadays, which is characterized by inadequate vitamin D exposure as well as by low calcium intake and excess phosphate intake [19, 32]. Based on the background information reported here, we assume that such a lifestyle may reduce levels of sCa and 25OHD or increase sPO levels, thus negatively affecting bone health in early life. There is no clear evidence, whether levels of the reported nutritional biomarkers refer to an impaired bone status in apparently healthy children.

Finally, there is insufficient evidence for the joint effect of these biomarkers combined with different PA behaviours such as habitual PA, WBE, and SED on bone health in early life.

In the IDEFICS study (Identification and prevention of dietary- and lifestyle- induced health effects in children and infants), bone stiffness index (SI) was assessed by quantitative ultrasound (QUS), which was used as an indicator for bone health in children. Furthermore, the IDEFICS study provides a comprehensive database of PA and nutritional parameters that may improve the understanding of the interplay between nutritional biomarkers, PA behaviour and bone health. The present nested case-control study (CCS) aimed to analyse the association of nutritional serum and urine biomarkers of the calcium-bone-homeostasis as well as of different PA behaviours such as habitual PA, WBE and SED with SI in preadolescent children having no bone-related diseases. Furthermore, we investigated the joint effects of these biomarkers combined with each PA behaviour on SI, and hypothesised that low levels of sCa, and 25OHD and high levels of sPO, uCa and uPO modify the association between PA, WBE, or SED and SI.

2. Methods

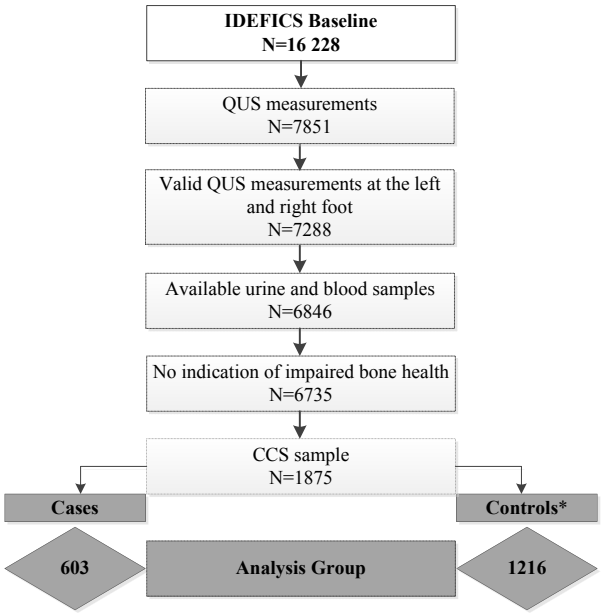
2.1 Study sample

This CCS was nested in the IDEFICS study, a population-based multicentre cohort study of children 2-9 years of age from eight European countries (Belgium, Estonia, Germany, Hungary, Italy, Spain, Sweden, and Cyprus). In the baseline survey (2007-2008), 16228 children were examined. The study was conducted according to the standards of the Declaration of Helsinki. All participating centres obtained ethical approval by their responsible authority. Participating children and their parents provided oral and written informed consent for all examinations and the storage of personal data and biological samples. The study design and examinations has been described previously [33, 34].

QUS was conducted in a subgroup of 7851 children with parental and own consent for this measurement. The nested CCS design was chosen to investigate the association of expensive biomarkers such as serum 25OHD with SI most efficiently by defining a group of cases and their randomly selected age-, sex- and country-matched controls for whom these markers are to be assessed. To date, there is lack of knowledge to define an age-, sex- and height-specific cut-off value in children that is based on a thorough risk assessment regarding various health outcomes like fractures. In order to define cases having a 'poor SI', we have used available criteria that define a pathological bone status in adults. On the one hand, we considered the World Health Organisation (WHO) criterion of osteopenia, which is defined as a bone mineral density (BMD) or bone mineral content (BMC) below one standard deviation (SD) of the young adult mean value (i.e. T-score < -1) [35]. On the other hand, we considered the SI T-score < -1 of the Achilles device, which is used as a referral criterion for a subsequent Dual-energy X-ray absorptiometry (DXA) measurement in adults [36, 37]. We are aware of the fact, that adult T-scores cannot be applied in children. Thus, the SI distribution has to be examined according to age, sex and growth [38]. The 15th age-, sex- and height-specific SI percentile corresponds to approximately –1 SD of the average SI. Children below the 15th age-, sex- and height-specific percentile value of SI were classified as cases having "poor SI". Controls were defined as children with an SI above or equal to the 15th age-, sex- and height-specific percentile value.

Figure 1 summarises how the children included in the CCS were selected. We considered all children from the IDEFICS baseline survey with available and valid QUS measurements on the left and right foot, available blood and urine samples as well as children without an

Figure 1. Inclusion criteria and selection of cases and controls forming the analysis group.



CCS - case-control study; QUS – quantitative ultrasonography

*56 controls were excluded because their matching stratum contained no cases.

indication of impaired bone health (i.e. without diseases or medical treatments affecting bone). To initiate laboratory analyses on additional blood parameters for this CCS (sCa, 25OHD, sPO) immediately after the baseline assessment, cases (below the 15th age- and sex-specific SI percentile) and randomly selected controls were drawn from the raw dataset (N=2020). Subsequent data cleaning steps, such as correction of implausible and erroneous values, as well as depleted and haemolytic serum samples led to a loss of subjects for the CCS resulting in 1875 potential cases or controls.

Cases were frequency matched to controls by age (two-year-age-groups), sex, and country. Cases with available accelerometer data were matched to controls having accelerometer data. Fifty-six controls had to be excluded from the analysis because their matching stratum contained no cases. The final CCS sample included 603 cases and 1216 controls, where 356 cases and 733 controls had available accelerometer data (Figure 1). Due to logistical reasons, laboratory analyses of sPO were delayed, which resulted in a lower number of available data on sPO in contrast to sCa or 25OHD (see Table 1).

2.2 Assessment of bone stiffness index

QUS measurements have been found to reflect strength and architecture of bone [39, 40]. In the IDEFICS study, QUS (Achilles Lunar Insight^{TM} GE Healthcare, Milwaukee, WI, USA) was used to assess SI of the right and the left calcaneus. SI was automatically calculated by

the raw parameters broadband ultrasound attenuation (dB/MHz) and speed of sound (m/s) [41]. The mean value was considered as a proxy for the bone status of the lower limbs and hip and to allow comparability with other studies [9]. To examine the reliability of Achilles devices, 60 children were repeatedly measured on either the right (N=30) or the left (N=30) calcaneus. Precision of Achilles measurements was calculated using the root-mean-square coefficient of variation (CV_{RMS}), expressed as a percentage, according to the Conference of Radiation Control Program Directors (CRCPD) Task Force on Bone Densitometry [42]. CV_{RMS} for SI was 9.2% on the right foot and 7.2% on the left foot.

In order to compare the SI measurements of the IDEFICS devices, we conducted a small reliability study in a convenience sample (N=91) based on five of the eight available QUS devices. Multilevel regression analysis showed a significant discrepancy between the devices for the absolute SI difference of the left and the right foot (unpublished data). Consequently, we controlled this discrepancy by limiting the absolute SI difference between SI of the left and the right calcaneus and excluded 3% of the QUS sample having the highest SI difference (97^{th} SI percentile: 45.26 SI difference). Further details about using QUS in the IDEFICS cohort have been described elsewhere [38, 43, 44].

2.3 Assessment of nutritional biomarkers

Whereas sCa (mmol/l), 25OHD (nmol/l), and sPO (mmol/l) were analysed in a preselected CCS sample only, uCa, uPO, and urine creatinine (uCr) were measured in morning urine samples of all IDEFICS children. 25OHD was manually analysed by radioimmunoassay (Immuno Diagnostics Systems, Frankfurt am Main, Germany). All other biomarkers were determined by photometric assay using Roche Integra 800 (Roche, Mannheim, Germany). Since no 24-hour urine was collected and to control for the concentration of the morning urine, uCa and uPO were related to the uCr as uCa/Cr ratio (mmol/mmol) and uPO/Cr ratio (mmol/mmol), respectively. The standardised procedures and analyses for the blood and urine samples in IDEFICS have been described elsewhere [45].

2.4 Assessment of physical activity behaviour

PA behaviour was assessed using objective and subjective methods. Habitual PA was assessed using accelerometry (Actigraph GT1M, ActiTrainer) in a subgroup of 9021 children, where parental consent for wearing an accelerometer was given. Data were considered to be valid when the child wore the accelerometer over three consecutive days for at least six hours per day. In the current analysis, 99.6% of the children had a total wearing time of at least 24 hours on all three days. We used accelerometer data with 60s epoch. Non-wearing time was

defined as 20 minutes or more of consecutive zero counts [46]. Accelerometers had to be taken off during water sports, showering, and sleeping. In order to reflect the average PA level during a day, we used average counts per minute (cpm). Additionally, MVPA (≥2296 cpm) and SED (≤100 cpm) were defined according to the cut-off values of Evenson [47]. We expressed MVPA and SED as the percentage (%) of total wearing time, to denote the duration of inactive time and MVPA per day.

Parents were asked "What kind of sport does your child do in a sports club?" This question offered four types of sports typical for each country and an open category to record sports that are not listed. The variable WBE was derived by categorising all types of sports into (i) WBE that includes sports with high-to-moderate mechanical loads on bone (ballgames, gymnastics, dancing, skating, martial arts, and athletics); (ii) no-impact WBE that includes sports with no or low mechanical loads (swimming, biking or horseback riding); and (iii) no sports. The latter includes children for whom no information on sports was available.

2.5 Assessment of confounding variables

Calcium intake was estimated by the habitual consumption of dairy products (milk, yoghurt, and cheese) as reported by parents in a food frequency questionnaire, which was developed and validated in the IDEFICS study [48, 49]. The response categories ranged from 'Never/less than once a week' up to 'Four or more times per day' and were converted into the weekly frequency of dairy product consumption.

Fat-free mass (FFM, kg), which is positively associated with different bone parameters [50], was calculated using the Tyrrell formula [51]. Here, the bioelectrical impedance and weight, which were measured with a bioelectrical impedance scale (Tanita scale BC420 MA) as well as height (Stadiometer SECA 225) were taken into account. Measured weight and height showed good intra- and inter-observer reliability within the IDEFICS cohort [52]. To control for extreme values, FFM values over 90% of a child's total body weight were not considered in this CCS analysis (N=6).

Less exposure to sunlight is associated with low 25OHD levels [2]. Because blood collection of cases and controls took place in different seasons, we adjusted for mean daylight duration (±0.1 hours) which was calculated for each examination month in each location, obtained from astronomical tables [53].

2.6 Statistical analyses

Odds ratios (OR) and 95% confidence intervals (95%CI) for poor SI were calculated using multivariate logistic regression conditional on age (continuous), sex, and country (7 dummy variables) to analyse the impact of each biomarker and each variable of PA behaviour within separate models. There is a lack of appropriate thresholds of these biomarkers and PA levels in relation to bone health in children. Therefore, we decided to categorize the continuous independent variables in order to allow simple interpretation, equally-sized groups and to detect potential threshold effects [54]. In a first step, we categorised continuous variables into tertiles (low, medium, and high) based on the distribution of the whole CCS analysis group. We presented the tertiles in the model as two dummy variables, with the suspected more favourable tertile as the reference. As categorization might lead to loss of statistical efficiency, we additionally performed trend tests using each independent variable continuously in the regression models.

In a second step, we assessed the associations of WBE, PA levels, MVPA and SED by different biomarker levels on poor SI. To maintain a sufficient number of participants in the groups, we dichotomised all continuous variables into low and high levels using the median of the whole CCS analysis group as cut-off value. In this study, low sCa (<2.5 mmol/l), low 25OHD (<43.0 nmol/l), high sPO (≥1.52 mmol/l), high uCa/Cr (≥0.29 mmol/mmol), and high uPo/Cr (≥4.28 mmol/mmol) were defined to be less favourable biomarker levels for SI. No sports, low PA levels (<595 cpm), low MVPA (<5.4%) and high SED (≥38.7%) were considered to be less favourable PA behaviours. For calculating OR for poor SI (four-group-comparison), the respective reference group was formed by children having more favourable biomarker levels combined with either WBE, high PA level, high MVPA or low SED. Additionally, we performed a sensitivity analysis to examine the association of less favourable PA behaviours combined with 25OHD levels below 30 nmol/l, as a cut-off value of vitamin D deficiency [2], with poor SI.

The statistical significance of the effect modification was tested on a multiplicative scale by adding an interaction term combining each biomarker with each variable of PA behaviour in separate multivariate logistic regression models.

Sensitivity analyses showed no major sex-differences of the osteogenic effects. As no major hormonal differences between the preadolescent boys and girls were suggested, we did not stratify by sex to retain statistical power.

All models were adjusted for FFM, daylight duration, and dairy product consumption (all continuous). Since PA was reported to counteract the adverse effect of SED on bone health [10], we additionally adjusted for MVPA (continuous) when examining the association between SED and SI. Statistical analyses were performed with SAS 9.3 (SAS Institute Inc., Cary, NC, USA).

3. Results

Table 1 presents the characteristics of the cases and controls. The SI of the children in the

Table 1. Characteristics of cases and controls.

	Cases		Controls	
	N (%)	mean (SD)	N (%)	mean (SD)
Sex	603 (100)		1216 (100)	
Male	317 (52.6)	-	643 (52.9)	-
Female	286 (47.4)	-	573 (47.1)	-
Age (years)	603 (100)	6.3 (1.7)	1216 (100)	6.3 (1.8)
Anthropometric measures	603 (100)		1216 (100)	
Bone stiffness index		62.0 (4.9)		78.7 (11.1)
Weight (kg)		23.4 (6.6)		23.7 (7.0)
Height (cm)		119.5 (12.0)		119.0 (12.0)
Fat-free mass (kg)	601 (99.7)	16.2 (4.3)	1199 (98.6)	16.3 (4.5)
Nutritional biomarkers				
Serum calcium (mmol/l)	536 (88.9)	2.5 (0.1)	1086 (89.3)	2.5 (0.1)
25OHD (nmol/l)	526 (87.2)	42.2 (18.6)	1063 (87.4)	45.7 (23.3)
Serum phosphate (mmol/l)	400 (66.3)	1.53 (0.14)	768 (63.2)	1.53 (0.14)
Urine Ca/Cr (mmol/mmol)	587 (97.3)	0.37 (0.28)	1191 (97.9)	0.36 (0.28)
Urine PO/Cr (mmol/mmol)	587 (97.3)	4.50 (1.61)	1191 (97.9)	4.47 (1.57)
Physical activity behaviour				
PA level (counts per minute)	356 (59.0)	593 (169)	733 (60.3)	620 (180)
Sedentary time (%)		39.7 (9.7)		39.2 (9.6)
Moderate-to-vigorous PA (%)		5.5 (3.0)		6.1 (3.3)
Wearing time of accelerometer (hours/week)		40.0 (11.2)		41.1 (11.6)
Weight-bearing exercise				
WBE	201 (33.3)	-	449 (36.9)	-
No-impact WBE	82 (13.6)	-	159 (13.1)	-
No sports	320 (53.1)	-	608 (50.0)	-
Potential confounding lifestyle factors				
Dairy product consumption (frequency per week)	600 (99.5)	21 (13)	1211 (99.6)	21 (12)
Daylight duration (hours/day)	603 (100)	11.0 (2.5)	1216 (100)	11.4 (2.5)
Country				
Italy	98 (16.3)	-	195 (16.0)	-
Estonia	39 (6.5)	-	118 (9.7)	-
Belgium	9 (1.5)	-	27 (2.2)	-
Sweden	71 (11.8)	-	141 (11.6)	-
Germany	272 (45.1)	-	431 (35.4)	-
Hungary	84 (13.9)	-	229 (18.8)	-
Spain	30 (5.0)	-	75 (6.2)	-

25OHD - 25-hydroxy-vitamin D_3; PA - physical activity; Ca/Cr - calcium/creatinine; PO/Cr - phosphate/creatinine; WBE - weight-bearing exercise

case group was approximately 17 units lower than that of the children in the control group. Whereas the average anthropometric measures, biomarker levels, and SED did not differ between the groups, cases had lower PA levels and MVPA than controls. In addition, the proportion of cases performing WBE was lower than that of controls (Table 1).

The subgroup with available accelerometer data did not differ from the full CCS analysis group with respect to age, sex, SI, weight, height, FFM, biomarkers, the variables of PA behaviour, dairy product consumption, daylight duration and country (data not shown).

Table 2a. Adjusted odds ratios (OR) and 95%-confidence interval (95%CI) for poor bone stiffness index by WBE, PA level, moderate-to-vigorous PA, and sedentary time.

Physical activity behaviour	Cases	Controls	OR[a] (95% Cl)
	N (%)	N (%)	
WBE			
Impact WBE	*198 (32.8)*	*445 (36.6)*	*1 (ref.)*
No-impact WBE	82 (13.6)	154 (12.7)	1.16 (0.83, 1.61)
No sports	318 (52.7)	596 (49.0)	**1.39 (1.08, 1.78)**
Missing	5 (0.8)	21 (1.7)	
PA level (counts per minute)			**0.85 (0.78, 0.93)**[b]
Tertile 3 (>668)	*104 (17.2)*	*259 (21.3)*	*1 (ref.)*
Tertile 2 (524 - 668)	126 (20.9)	235 (19.3)	**1.57 (1.12, 2.19)**
Tertile 1 (<524)	125 (20.7)	232 (19.1)	**1.85 (1.28, 2.68)**
Missing	248 (41.1)	490 (40.3)	
Moderate-to-vigorous PA (%)			**0.91 (0.87, 0.95)**[c]
Tertile 3 (>6.7)	108 (17.9)	253 (20.8)	*1 (ref.)*
Tertile 2 (4.2 - 6.7)	111 (18.4)	248 (20.4)	1.16 (0.83, 1.61)
Tertile 1 (<4.2)	135 (22.4)	224 (18.4)	**1.69 (1.20, 2.40)**
Missing	249 (41.3)	491 (40.4)	
Sedentary time (%)			1.08 (0.84, 1.24)[c]
Tertile 1 (<34.6)	*116 (19.2)*	*242 (19.9)*	*1 (ref.)*
Tertile 2 (34.6 - 42.8)	121 (20.1)	237 (19.5)	0.98 (0.69, 1.37)
Tertile 3 (>42.8)	117 (19.4)	246 (20.2)	0.86 (0.57, 1.30)
Missing	249 (41.3)	491 (40.4)	

PA - physical activity; ref. – reference group; WBE - weight bearing exercise

[a] OR conditional on age, sex, and country, and adjusted for fat-free mass (kg), daylight duration (hours/day), and consumption of dairy products (frequency/week). OR for sedentary time was additionally adjusted for moderate-to-vigorous PA

[b] OR per 100 counts per minute (1 unit refers to 0.01 counts per minute)

[c] OR per 100% sedentary time and 100% moderate-to-vigorous PA (1 unit refers to 0.01%)

Tables 2a and 2b present adjusted OR and 95% confidence intervals of poor SI by a) WBE, PA level, MVPA, and SED and b) by nutritional biomarkers. The OR for poor SI was increased in children not performing sports compared to children performing WBE. We also observed increased ORs for medium and low PA levels compared to high PA levels as well as for low MVPA compared to high MVPA. No increased OR for poor SI was observed for high SED (Table 2a). For low sCa, low 25OHD and high uCa the ORs for poor SI tended to be moderately elevated but were not statistically significant (Table 2b).

Table 2b. Adjusted odds ratios (OR) and 95% confidence intervals (95%CI) for poor bone stiffness index by nutritional biomarkers.

Biomarkers	Cases	Controls	OR[a] (95% Cl)
	N (%)	N (%)	
Serum calcium (mmol/l)			0.57 (0.18-1.77)
Tertile 3 (>2.5)	*157 (26.0)*	*357 (29.4)*	*1 (ref.)*
Tertile 2 (=2.5)	217 (36.0)	421 (34.6)	1.15 (0.88-1.50)
Tertile 1 (<2.5)	159 (26.4)	289 (23.8)	1.27 (0.94-1.70)
Missing	70 (11.6)	149 (12.3)	
25OHD (nmol/l)			0.95 (0.88-1.01)[b]
Tertile 3 (>50.7)	*153 (25.4)*	*376 (30.9)*	*1 (ref.)*
Tertile 2 (34.9 - 50.7)	170 (28.2)	350 (28.8)	1.05 (0.79-1.41)
Tertile 1 (<34.9)	200 (33.2)	319 (26.2)	1.24 (0.89-1.72)
Missing	80 (13.3)	171 (14.1)	
Serum phosphate (mmol/l)			1.04 (0.94-1.14)[c]
Tertile 1 (<1.45)	*138 (22.9)*	*250 (20.6)*	*1 (ref.)*
Tertile 2 (1.45 - 1.55)	83 (13.8)	140 (11.5)	1.12 (0.78-1.61)
Tertile 3 (>1.55)	176 (29.2)	359 (29.5)	0.94 (0.69-1.27)
Missing	206 (34.2)	467 (38.4)	
Urine Ca/Cr (mmol/mmol)			1.37 (0.94-2.00)
Tertile 1 (<0.19)	*191 (31.7)*	*399 (32.8)*	*1 (ref.)*
Tertile 2 (0.19 - 0.42)	189 (31.3)	391 (32.2)	1.00 (0.78-1.29)
Tertile 3 (>0.42)	202 (33.5)	380 (31.3)	1.20 (0.93-1.55)
Missing	21 (3.5)	46 (3.8)	
Urine PO/Cr (mmol/mmol)			1.04 (0.97-1.12)
Tertile 1 (<3.70)	*202 (33.5)*	*387 (31.8)*	*1 (ref.)*
Tertile 2 (3.70 - 4.94)	182 (30.2)	397 (32.6)	0.85 (0.66-1.10)
Tertile 3 (>4.94)	198 (32.8)	386 (31.7)	1.07 (0.82-1.39)
Missing	21 (3.5)	46 (3.8)	

25OHD - 25-hydroxy-vitamin D_3; ref. – reference group; Ca/Cr - calcium/creatinine; PO/Cr - phosphate/creatinine

[a] OR conditional on age, sex, and country, and adjusted for fat-free mass (kg), daylight duration (hours/day), and consumption of dairy products (frequency/week)

[b] OR per 10 nmol/l 25OHD (1 unit refers to 0.1 nmol/l 25OHD)

[c] OR per 0.1 mmol/l serum phosphate (1 unit refers to 10 mmol/l serum phosphate)

The tests for effect modifications of the biomarkers on the association between SI and WBE, PA level, MVPA or SED were statistically non-significant (data not shown). Results for the four-group-comparison, i.e. the OR for WBE, PA levels, MVPA and SED stratified by more favourable versus less favourable serum biomarker levels are presented in table 3a. We observed increased OR in children performing no sports or low MVPA and having either less favourable or more favourable levels of serum biomarkers compared to children in the respective reference group. A similar pattern in terms of serum biomarkers was observed amongst children having low PA levels. The ORs however were lower than for low MVPA and no sports. We did not observe an association between SED and SI, neither for less favourable nor for more favourable biomarker levels (Table 3a).

Table 3b presents the ORs for WBE, PA levels, MVPA and SED stratified by more favourable and less favourable urine biomarker levels. For all less favourable PA behaviours, except for SED, we observed increased OR for poor SI in children having either less favourable or more favourable uCa/Cr or uPO/Cr. The OR were not elevated for high SED neither combined with less favourable nor combined with more favourable uCa/Cr or uPO/Cr levels.

Table 3a. Odds Ratios (OR)[a] and 95% confidence intervals for poor bone stiffness index by more favourable and less favourable levels of serum biomarkers in combination with WBE, PA levels, moderate-to-vigorous PA, and sedentary time.

PA behaviour / Serum biomarkers		WBE			PA level (counts per minute)		Moderate-to-vigorous PA (%)		Sedentary time (%)	
		WBE	**No-impact WBE**	**No sports**	**≥595**	**<595**	**≥5.4**	**<5.4**	**<38.7**	**≥38.7**
Serum calcium (mmol/l)	**≥2.5**	*1 (ref.)*	1.24 (0.81, 1.89)	**1.43 (1.06, 1.94)**	*1 (ref.)*	1.28 (0.90, 1.82)	*1 (ref.)*	**1.44 (1.02, 2.02)**	*1 (ref.)*	0.83 (0.57, 1.23)
	<2.5	1.09 (0.72-1.63)	1.34 (0.74, 2.42)	**1.77 (1.22, 2.59)**	1.04 (0.68-1.60)	**1.62 (1.03, 2.55)**	0.88 (0.59, 1.32)	**1.90 (1.24, 2.91)**	0.98 (0.63, 1.52)	1.05 (0.64, 1.72)
25OHD (nmol/l)	**≥43.0**	*1 (ref.)*	1.51 (0.89, 2.56)	**1.83 (1.21, 2.76)**	*1 (ref.)*	1.30 (0.85, 1.97)	*1 (ref.)*	**1.64 (1.08, 2.48)**	*1 (ref.)*	0.88 (0.55, 1.39)
	<43.0	**1.55 (1.03, 2.33)**	1.56 (0.91, 2.68)	**1.90 (1.28, 2.83)**	1.23 (0.79, 1.89)	**1.70 (1.08, 2.65)**	1.37 (0.87, 2.13)	**1.89 (1.21, 2.97)**	1.19 (0.77, 1.85)	1.12 (0.69, 1.80)
Serum phosphate (mmol/l)	**<1.52**	*1 (ref.)*	1.54 (0.85, 2.80)	**1.82 (1.16, 2.86)**	*1 (ref.)*	1.39 (0.82, 2.35)	*1 (ref.)*	1.24 (0.74, 2.05)	*1 (ref.)*	0.97 (0.57, 1.65)
	≥1.52	1.33 (0.85, 2.08)	1.24 (0.68, 2.29)	**1.75 (1.13, 2.71)**	1.00 (0.63, 1.60)	1.17 (0.72, 1.90)	0.83 (0.52, 1.31)	1.23 (0.77, 1.97)	1.11 (0.69, 1.77)	0.73 (0.43, 1.24)

25OHD - 25-hydroxy-vitamin D_3; PA - physical activity; ref. – reference group; WBE - weight-bearing exercise

[a] OR conditional on age, sex, and country, and adjusted for fat-free mass (kg), daylight duration (hour/day), and consumption of dairy products (frequency/week). OR for sedentary time was additionally adjusted for moderate-to-vigorous PA

Table 3b. Odds Ratios (OR)[a] and 95% confidence intervals for poor bone stiffness index by more favourable and less favourable levels of urine biomarkers in combination with WBE, PA levels, moderate-to-vigorous PA, and sedentary time.

PA behaviour / Urine biomarkers		WBE			PA level (counts per minute)		Moderate-to-vigorous PA (%)		Sedentary time (%)	
		WBE	**No-impact WBE**	**No sports**	**≥595**	**<595**	**≥5.4**	**<5.4**	**<38.7**	**≥38.7**
Urine Ca/Cr (mmol/mmol)	**<0.29**	*1 (ref.)*	**1.74 (1.10, 2.75)**	**1.67 (1.19, 2.33)**	*1 (ref.)*	1.32 (0.89, 1.96)	*1 (ref.)*	**1.52 (1.03, 2.25)**	*1 (ref.)*	0.86 (0.56, 1.32)
	≥0.29	**1.61 (1.14, 2.28)**	1.22 (0.75, 1.98)	**1.86 (1.31, 2.62)**	1.21 (0.83, 1.77)	**1.48 (1.00, 2.19)**	1.12 (0.76, 1.66)	**1.75 (1.19, 2.58)**	1.13 (0.77, 1.66)	1.02 (0.66, 1.58)
Urine PO/Cr (mmol/mmol)	**<4.28**	*1 (ref.)*	1.27 (0.80, 2.02)	**1.51 (1.09, 2.10)**	*1 (ref.)*	**1.64 (1.10, 2.44)**	*1 (ref.)*	1.43 (0.91, 2.10)	*1 (ref.)*	1.00 (0.65, 1.55)
	≥4.28	1.13 (0.79, 1.61)	1.19 (0.74, 1.90)	1.42 (0.99, 2.03)	1.13 (0.77, 1.66)	1.12 (0.74, 1.69)	0.78 (0.52, 1.16)	1.32 (0.89, 1.95)	0.99 (0.67, 1.47)	0.75 (0.48, 1.18)

PA - physical activity; Ca/Cr - calcium/creatinine ratio; PO/Cr - phosphate/creatinine ratio; ref. – reference group; WBE - weight-bearing exercise

[a] OR conditional on age, sex, and country, and adjusted for fat-free mass (kg), daylight duration (hour/day), and consumption of dairy products (frequency/week). OR for sedentary time was additionally adjusted for moderate-to-vigorous PA

4. Discussion

This is the first study assessing the association of nutritional biomarkers in combination with WBE, habitual PA, MVPA and SED with SI in preadolescent children having no bone-related diseases.

Our results highlight the detrimental association of low PA and a lack of sports with SI, whereas levels of nutritional serum and urine biomarkers were not strongly associated. Furthermore, the levels of each biomarker did not appear to modify the association of WBE, PA level, MVPA and SED with SI. However, our data suggest that more favourable levels of sCa, uCa, 25OHD or sPO combined with high-impact PA seem to be most beneficial for bone health.

In agreement with our findings, only a few studies have reported a beneficial effect of high PA levels or WBE combined with high calcium intake or high 25OHD on skeletal development in early life, in which neither WBE nor high calcium or high 25OHD alone induced a greater bone gain [11-15, 55]. In a cross-sectional study, Rowland et al. observed interaction effects of vigorous PA and calcium intake on bone in 8-11-year-old children [15]. Furthermore, intervention studies reported the greatest bone mineral acquisition after 6 months of calcium supplementation and additional WBE in pre- and early adolescent girls and boys [12-14]. Regarding vitamin D, a cross-sectional study of 12.5-17.5-year-old Spanish adolescents reported the highest BMC in subjects with sufficient 25OHD and being active at least 60 minutes a day [55]. Frost's theorem supports the findings from these studies stating that the mechanical demands of high-impact PA forces the bone to adapt. This bone adaption may require adequate calcium intake and thus high sCa and 25OHD levels [17, 56].

The lack of an association between high SED and poor SI which we observed is possibly due to a confounding effect of MVPA. Similar observations have also been reported in previous studies [10]. In a pre-analysis, we examined the association of SED and SI without adjusting for MVPA and observed elevated odds for poor SI (data not shown). Thus, our findings highlight the prominent role of high PA levels, MVPA and impact WBE on bone health in children. Nevertheless, data on the adverse effect of SED on bone health in children, especially in combination with nutritional biomarkers are scarce. Only one study in 6-12-year-old Flemish children did not observe any interaction between SED and dairy consumption in terms of their effect on SI, which is in line with our findings [9].

Our findings do not support a modifying effect of nutritional biomarker levels on the association between PA or WBE and SI, but they indicate a slightly increased risk for poor SI

in children with less favourable sCa, 25OHD, and uCa levels. Here, it should be considered, that sCa and sPO values in our study varied within normal ranges [57, 58]. Furthermore, 72% of the cases and 79% of the controls had 25OHD values above the threshold of vitamin D deficiency (≥30 nmol/l) [2]. According to the reference values of uCa and uPO for 7-9-year-old children from Slev et al., respectively 98% and 92% of our CCS children were below the 95th percentile and may be considered in a normal range [59].

Serum calcium is known to be tightly maintained within a narrow normal range (2.2-2.6 mmol/l) and is controlled by its strong homeostasis interacting with bone. That is, once sCa levels decrease, PTH levels increase to release calcium from the bone in order to maintain calcium homeostasis [17]. Thus, it may be considered unlikely that significant associations between sCa and SI will be observed, especially when sCa varies in a normal range. As such, PTH, 25OHD or uCa may be better biomarkers for detecting impaired bone mineralization at an early stage.

To what extent 25OHD and uCa in normal ranges may reflect impaired bone mineralization remains questionable. Although our results did not reveal strong associations between these biomarkers and poor SI, some studies observed reduced bone indices in children with serum biomarkers and uCa values within normal ranges [24-26, 60]. For instance, Shi et al. reported that long-term increased uCa excretion within a physiologically normal range may already predict reduced BMC and BMD in healthy children [60]. Furthermore, bone demineralisation processes have already been observed at 25OHD levels close to 50 nmol/l, while cut-off values of vitamin D deficiency in children vary between 20 and 37.5 nmol/l of 25OHD [2, 23, 32]. In order to consider potential adverse effects of vitamin D deficiency (<30 nmol/l) with SI in our study, our sensitivity analysis using this cut-off value did not reveal remarkably higher ORs compared to those obtained with our actual cut-off value (<43 nmol/l). It may still be noted that the ORs for poor SI were elevated when examining the association of less favourable PA behaviours combined with 25OHD levels below 30 nmol/l. Although our cut-off values may not indicate physiologically insufficient or deficient biomarker levels, lower sCa and 25OHD levels may serve as indicators to detect imbalances of calcium homeostasis, and higher uCa may indicate increased bone resorption already at an early stage before an impaired skeletal development occurs [17, 18]. The role of increased, i.e. less favourable sPO and uPO for bone health remains unclear due to the lack of evidence in healthy children [28, 30]. Unexpectedly, our findings indicate an increased risk for poor SI in children having low uPO excretion. In light of these findings, a single threshold may be inadequate to define a sufficient or insufficient level of these biomarkers for bone health, particularly in growing

children. Thus, the definitions of normal ranges of nutritional biomarkers in terms of inadequate bone mineralisation or bone-related diseases in children remain heterogeneous and controversial [2, 23, 57-59, 61].

Interestingly, we observed no differences in FFM between cases and controls. It may thus be concluded that FFM is not associated with SI. Previous studies reported a positive association of FFM with bone indices, which is mostly used as an indicator for muscle mass [50, 62]. A post-hoc analysis on the association of FFM with SI in our CCS revealed positive associations by showing increased ORs for poor SI for children in the first (OR=2.19, 95%CI 1.44-3.34) and second tertile of FFM (OR=1.50, 95%CI 1.13-1.99) compared to children in the third tertile. In addition, a previous published analysis within the IDEFICS study confirms the positive association between FFM and SI in school children (6-9 years, N=4149) [43]. Another recent analysis of preschool (2-<6 years, N=1539) and school children (6-10 years, N=2982) indicated a positive association of FFM with SI only in school children, while a negative association was observed in preschool children (unpublished data).

A strength of our study is the assessment of SI, of objectively measured PA, as well as of serum and urine biomarkers in a large sample of children.

However, as no data were available on the children's fracture history in the IDEFICS study, as recommended from the International Society of Clinical Densitometry (ISCD) [63], we cannot say whether our threshold value (<15th age-, sex- and height-specific SI percentile) indicates a true 'poor SI' and thus impaired bone mineralization. The lack of knowledge on the predictive value of SI measurements for determining skeletal fragility or fracture risk in children further limits the comparison of our results with those of previous studies. Only a few studies, which compared QUS with x-ray measurements in children, give some insights on whether SI measurements indicate bone health. However, the studies reported heterogeneous results [36, 39, 41, 64, 65]. For instance, the results of a sub-study within the IDEFICS project indicated negative correlations between SI and BMD in 37 4-8-year-old children [64]. In contrast, Sundberg et al. reported positive correlations between SI and BMD in 11-16-year-old children (N=280). Nevertheless, they reported that only 41-49% of the children in the lowest quartile for SI were also identified in the lowest quartile for BMD [65]. Jaworski et al. also detected good correlations in 6-13-year-old children (N=89) and reported significant lower SI values in 18 osteopenic children (average SI: 50.6±13.7) compared to their 71 healthy controls (average SI: 72.7±12.0) [39]. In addition to the few observations made in children, the sensitivity of SI measurements for identifying osteoporotic

postmenopausal women diagnosed with DXA was observed to be low. Nevertheless, nowadays SI is used for predicting the risk of osteoporotic fractures in adults [36, 66]. Finally, it should be kept in mind, that QUS and x-ray measurements give different information on bone characteristics [41]. Thus, the relevance of the 15th SI percentile as an indicator for 'poor SI' and thus for poor bone health in children needs to be confirmed in future prospective trials, especially including children with a clinical significant fracture history.

We are aware of the limitations regarding the categorisation of all continuous variables and thus the smaller sample sizes in the categories and the loss of information. Therefore, we also conducted sensitivity analyses considering all variables as continuous. We did not observe any modifying effects of the nutritional biomarkers on the association between PA behaviour and SI (results not shown), which conforms to our findings when considering categorical variables. Thus, we present our findings as categorized; making them easier to interpret and understand [54].

Another limitation is the reported information on sports activities using a non-validated questionnaire. However, our findings are in line with previous studies that observed a beneficial osteogenic effect of WBE [1, 5].

Pubertal status was not assessed. Nevertheless, we have to assume that the majority of our children aged 2-<10 years were preadolescent.

In conclusion, our study extends the knowledge of the association between SI and high-impact PA, known as a protective factor for bone health in children. In addition, we provide the first evidence that the adverse association between low PA levels or no sports with SI is not or is only modestly modified by markers of the calcium-bone-homeostasis such as calcium, 25OHD, and phosphate. In contrast, previous studies have suggested that sufficient calcium intake and 25OHD may be needed during high-impact PA or WBE to allow an adequate bone adaption. However, monitoring circulating calcium or 25OHD may help to detect their deficits in time and to apply early intervention strategies regarding diet and PA to ensure an optimal skeletal development.

Disclosure

The authors state that they have no conflicts of interest.

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Appendix D

Reference Values of Bone Stiffness Index and C-Terminal Telopeptide in Healthy European Children

Herrmann D, Intemann T, Lauria F, Mårild S, Molnár D, Moreno LA, Sioen I, Tornaritis M, Veidebaum T, Pigeot I, Ahrens W, and on behalf of the IDEFICS consortium

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ORIGINAL ARTICLE

Reference values of bone stiffness index and C-terminal telopeptide in healthy European children

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BACKGROUND/OBJECTIVE: Quantitative ultrasound measurements and bone metabolic markers can help to monitor bone health and to detect impaired skeletal development. Population-based reference values for children may serve as a basis for preventive measures to reduce the risk of osteoporosis and osteoporotic fractures in later life. This is the first paper providing age-, sex- and height-specific reference values for bone stiffness index (SI) and serum carboxy-terminal cross-linking telopeptide of type I collagen (CTX) in healthy, apparently prepubertal children.

SUBJECTS/METHODS: In the population-based IDEFICS baseline survey (2007–2008) and follow-up (2009–2010), 18 745 children from eight European countries were newly recruited. A total of 10 791 2–10.9-year-old and 1646 3–8.9-year-old healthy children provided data on SI of the right and left calcaneus and serum CTX, respectively. Furthermore, height and weight were measured. Percentile curves were calculated using the General Additive Model for Location Scale and Shape (GAMLSS) to model the distribution of SI and CTX depending on multiple covariates while accounting for dispersion, skewness, and the kurtosis of this distribution.

RESULTS: SI was negatively associated with age and height in children aged 2–5 years, whereas a positive association was observed in children aged 6–10 years. The dip in SI occurred at older age for higher SI percentiles and was observed earlier in taller children than in smaller children. The CTX reference curves showed a linear-positive association with age and height. No major sex differences were observed for the SI and CTX reference values.

CONCLUSION: These reference data lay the ground to evaluate bone growth and metabolism in prepubertal children in epidemiological and clinical settings. They may also inform clinical practice to monitor skeletal development and to assess adverse drug reactions during medical treatments.

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INTRODUCTION

Unhealthy lifestyle habits, like physical inactivity may hamper the child attaining its genetically programmed peak bone mass. Moreover, an impaired skeletal development in early life can be caused by an underlying genetically or lifestyle-related disorder, for example, adiposity as well as by certain medical treatments.[1–4] This may in return increase the risk of osteoporosis and osteoporotic fractures later in life.[5–7] Previous studies reported that children having a low bone mineral density in prepuberty remain to have a low bone mineral density in adolescence.[8–10] In contrast, other studies showed that an impaired skeletal development during childhood may be largely reversible when treating underlying diseases.[11–13]

Age- and sex-specific bone reference values can help to assess skeletal development during childhood and to compare the bone health status of a child with that of a healthy population having the same age, sex and ethnicity. Thus, deviations from a normal skeletal development may be identified in an early stage. Until now, paediatric reference data have mainly been used for diagnosing poor bone acquisition in children who are suffering from multiple fractures, diseases or receiving medical treatments affecting bone metabolism.[11,14] Bone reference data may be not only a diagnostic tool for children with bone diseases[15] but may also serve to monitor apparently healthy subjects and to identify those who may benefit from specific interventions to improve bone health.

Only few studies established paediatric reference values for physical bone measurements. Reference values are mainly reported for bone mineral content and areal bone mineral density (aBMD) assessed with dual-energy X-ray absorptiometry (DXA). These reference data are mostly based on Asian, mixed American and Caucasian populations and are predominantly available for children above the age of 6 years.[10,14,16–21] Several studies reported limitations of DXA to assess bone status in growing individuals. Especially, the use of aBMD results in an artificial underestimation of the bone status in short people and an overestimation in tall people due to its areal but not volumetric measurement. This measure is size dependant owing to its two-dimensional image projection of a three-dimensional structure. That is, it does not account for depth or size and hence, it is not able to predict the 'true density' of the bone.[16,22] Furthermore, DXA does not distinguish between trabecular and cortical bone. It provides only little information on bone geometry and trabecular microarchitecture. Moreover, there is no evidence that aBMD is predictive for fracture risk in children.[23]

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Quantitative ultrasound (QUS) may be a more appropriate method to monitor skeletal development in children compared to DXA. QUS is a quick, cost-efficient and radiation-free method to evaluate bone stiffness that indicates density, structure and composition of the bone.[24,25] Especially, the calcaneus was found to be a reliable location to assess bone status. The calcaneus consists of 90% trabecular bone that shows a high metabolic rate. The bone microarchitecture is similar to that of the lumbar spine and the femoral neck, which are major body sites for diagnosing osteoporosis.[24] Besides the difference between QUS and DXA measurements assessing bone status, bone stiffness showed good correlations with aBMD assessed by DXA in adults[25] and children.[26,27] Furthermore, it revealed to be highly prognostic with regard to bone fractures in adults[28] and proved to be a valid measure for the risk for osteopenia in young patients.[27] Up to now, QUS references were established mainly for adults but are barely available for children and adolescents.[20,24]

According to the recommendation of the International Society for Clinical Densitometry, a combined assessment of physical measures and biochemical bone metabolic markers is useful to identify a reduced skeletal development in children.[11,15,29] Physical bone measurements predict the actual bone status. Bone metabolic markers, such as osteocalcin or collagen cross-linked telopeptides indicate reduced or increased bone turnover processes. These clinical parameters may be useful to monitor skeletal development, as they can be repeated at much shorter intervals. Hence, they allow detection of acute effects caused by diseases or treatments long time before changes in bone mass occur.[15] Reference data on these bone metabolic markers are rare in children and are mostly limited to selected clinical populations with a limited age range.[11,15,30,31]

In the IDEFICS (Identification and prevention of Dietary- and lifestyle-induced health Effects in Children and InfantS) study, bone stiffness index (SI) was measured using QUS in a large European child cohort. Serum carboxy-terminal cross-linking telopeptide of type I collagen (β-CrossLaps, CTX) as an indicator for bone resorption and bone turnover was measured in a case-control subsample.

The aim of the present study was to provide age- and sex-specific reference values for SI and CTX adjusted for body size for apparently prepubertal children based on an European sample of 18 745 healthy children who participated in the IDEFICS study. To calculate reference values and percentile curves, the General Additive Model for Location Scale and Shape (GAMLSS) was used to model the distribution of SI and CTX depending on multiple covariates while accounting for dispersion, skewness, and particularly the kurtosis of this distribution.[32,33]

MATERIALS AND METHODS

Analysis group

In the population-based IDEFICS baseline survey (2007–2008, T_0) and follow-up (2009–2010, T_1) 18 745 children aged 2–10.9 from eight European countries (Belgium, Cyprus, Estonia, Germany, Hungary, Italy, Spain, Sweden) were newly recruited and examined. The IDEFICS study is one of the current largest prospective European child cohorts.[34] Besides the comprehensive IDEFICS examination programme, heel QUS was applied in a subsample.

Written informed consent was obtained from the parents and each child was asked to give verbal assent immediately before examination. Participants were free to omit specific modules like blood drawing. In each country, the participating centres obtained ethical approval by their responsible authority. The whole examination programme of the IDEFICS study as well as further information regarding the study design were recently described in detail.[34–36]

Figure 1 summarises all exclusion criteria as well as the number of included and excluded children of the final SI analysis group. A total of 11 414 children having their first QUS measurement in T_0 ($N = 7539$) or having no QUS in T_0 but in T_1 ($N = 2714$) as well as all newly recruited children with QUS measurement in T_1 ($N = 1161$) were considered for the current analysis. Thirty-three children from T_0 having no QUS in T_0 but in T_1 had an age above 10.9 years at T_1 and were therefore excluded from the present analysis.

We conducted a small reliability study based on five of eight Achilles devices that were used within the IDEFICS study to investigate measurement errors and outliers of SI values. QUS measurements were performed in 91 subjects and repeated three times per foot and device. Accounting for repeated measurements, a multilevel regression was performed, but showed no differences in SI values between the three repeated measurements and between measurements at the left and right foot. However, a significant deviation of the SI values between the devices was observed varying within a range of 0–5 SI units on average. A significant discrepancy between the devices was also found for the absolute SI difference of the left and right foot. Based on these results, the 97th percentile of the absolute difference between the SI values of the right and left foot was considered to reflect unreliable measurements. According to this quality criterion, 341 children were excluded from the present analysis. Decreased birth weight associated with premature birth and growth retardation causes reduced bone accretion. Hence, 14 children with a birth weight < 1000 g were excluded according to the ICD-10 criterion on 'extremely low birth weight'.[37,38] Moreover, diseases and medical treatments that directly affect bone metabolism were identified by a health and medical history questionnaire. Diseases and treatments were categorized according to the ICD-10 and ATC coding, respectively (see Table 1).[37,39] We excluded 111 children with a disease and 115 children receiving medical treatments that directly impair bone metabolism.[5,15] Finally, nine children were excluded because their measurement values exceeded five s.d. values of the age- and sex-specific height *z*-score according to International Obesity Task Force.[40] Thus, the final analysis group consisted of 10 791 children for SI (see Figure 1).

Besides other bone metabolic markers, serum CTX has been analysed in a subsample drawn from the IDEFICS cohort for a case-control study. SI was chosen as an indicator for bone health. Within this case-control study, children between 2 and 9.9 years who had an SI below the 15th age- and sex-specific percentile of all IDEFICS children were eligible and classified as cases having low SI. Controls were randomly selected from the remaining cohort and matched to cases by age, sex and study centre. A total of 1646 children aged 3.0–8.9 years provided sufficient data on CTX and SI and were considered for the CTX analysis group, applying the same exclusion criteria as for the SI analysis group (see Table 1 and Figure 2).

Measurements

QUS (Lunar Achilles Insight, GE Healthcare, Milwaukee, WI, USA)[41] was used to measure SI on the right and left foot. Both measurements were used to calculate the mean, which was considered for calculating the present reference values. In a subsample, the raw QUS measurements broadband ultrasound attenuation and speed of sound were measured. Reference values of these parameters will be considered in further publications. For standardised measurements, the child was asked to sit barefoot on a stable chair directly in front of the device. The leg was positioned so that the foot and calf were aligned with the foot positioner. The foot was positioned using an adapter for children's feet to put the calcaneus in focus. The study nurse made sure that the foot was flat and positioned firmly against the bottom of the footplate and that the child was not moving during the measurement. When changing the measured foot, the position on the chair had to be adjusted. Quality control was performed weekly, using the implemented quality assurance test of Achilles Insight.[41]

For laboratory analysis of CTX, blood samples were drawn in the morning (between 8 a.m. and 10 a.m.) after an overnight fast. Blood samples were centrifuged (2100 *g*, 10 min, room temperature) within 2 h of venipuncture, frozen within about 4 h after withdrawal and stored at -80 °C. Serum CTX (ng ml^{-1}) was determined by electrochemiluminescence assay using Roche, ECLIA Modular E17 (Roche, Diagnostics, Mannheim, Germany).

Body height (Seca 225 stadiometer, seca, Birmingham, UK) and body weight (Tanita BC 420 SMA, Tanita Europe, Sindelfingen, Germany) were measured to the nearest 0.1 cm and 0.1 kg, respectively. Body mass index (kg m^{-2}) as well as *z*-scores of height, weight and body mass index were calculated according to the British 1990 growth reference.[40] Moreover, children were categorised as thin (grade 1–3), normal weight, overweight and obese using the International Obesity Task Force criteria.[42] Leg-to-leg bioelectrical impedance was measured with the Tanita scale and used to calculate fat-free mass based on the Tyrrell formula.[43] fat-free mass index (kg m^{-2}) was calculated according to Wells and Cole.[44]

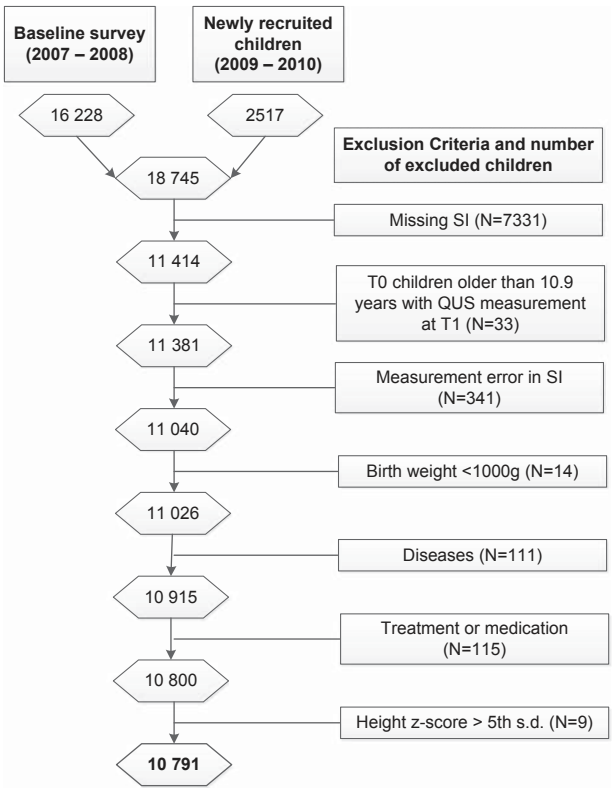

Figure 1. Number of included and excluded children per exclusion criteria for the SI analysis group from the IDEFICS population.

Statistical analyses

For both analysis groups, mean and s.d. of SI and CTX as well as of age, height and weight of 1-year age groups were calculated to describe study characteristics. In particular, percentile values of SI and CTX were calculated as a function of different covariates, for example, age, height or weight, stratified by sex using the GAMLSS method.

Due to the case-control design, the CTX sample included a high percentage (50%) of cases with a low SI that was defined below the 15th age- and sex-specific SI percentiles. To correct the GAMLSS analyses for oversampling of cases against controls, we used weights for cases and controls in the GAMLSS models calculating percentile values for CTX. These weights were based on the original distribution in the study sample (cases: 15%; controls: 85%).

The GAMLSS method is an extension of the LMS method that models three parameters depending on one explanatory variable: (M) accounts for the median of the outcome variable and the coefficient of variation; (S) accounts for the variation around the mean and adjusts for non-uniform dispersion, while the skewness (L) accounts for the deviation from a normal distribution using a Box–Cox transformation. The GAMLSS method is able to consider distributions with varying kurtosis and multiple covariates in the model. We used the *gamlss* package (version 4.2–6) of the statistical software R (version 3.0.1).[45] Different distributions, that is, the Box–Cox power exponential, the Box–Cox transformation or the Box–Cox Cole and Green distribution were fitted to the observed distribution of SI. The normal, the power exponential and the *t*-family distribution were fitted to the observed and weighted distribution of CTX.

Depending on the potential covariates (for example, age, height, height *z*-score, weight, weight *z*-score, body mass index, body mass index *z*-score and fat-free mass index), distribution parameters were modelled either as a constant, as a linear function or as a cubic spline. To identify the best combination of these covariates, different combinations were investigated to choose the best model. Goodness of fit for both dependant variables SI and CTX was assessed based on the Bayesian Information Criterion, Q–Q plots and worm plots.[46] The best model for SI and CTX included the covariates, age and height. For modelling SI percentiles, no adjustment for kurtosis was needed. Therefore, the final SI model for both sexes considered a Box–Cox Cole and Green distribution modelling μ as a linear function of age and a cubic spline of height, log(σ) and ν as cubic splines of height only. The final model for CTX considered a normal distribution modelling μ as a linear function of age and height and log(σ) as constant in both sexes. Figure 3, in which the Q–Q plots of residuals for SI and CTX in boys are depicted, shows that the derived percentiles fit the data well. The same was observed for girls (data not shown).

Final models of SI and CTX were used to calculate percentile values for the 1st, 3rd, 10th, 25th, 50th, 75th, 90th, 97th and 99th percentiles.[33,45] To present values of the calculated percentiles, tables were presented for 1-year age groups and seven height percentiles (3rd, 10th, 25th, 50th, 75th, 90th and 97th). Further age- and sex-specific reference values of the 5th, 15th, 85th and 95th percentiles of SI and CTX will be provided in (Supplementary Tables A–D). In the following, children aged 2– < 6 years and 6– < 11 years will be referred to as preschool and primary school children, respectively.

Table 1. Frequency of excluded disorders and medical treatments for establishing the SI and CTX analysis group

	Excluded children (N) for SI	*Excluded children (N) for CTX*
Disease		
Cancer	13	2
Disorders of thyroid gland	13	1
Endocrine disorders (for example, lack of growth hormone)	8	—
Vitamin D deficiency /rickets /osteomalacia	1	—
Dietary calcium deficiency	1	—
Cerebral palsy	12	2
Hemiplegia	1	—
Rheumatic fever	6	1
Disorders of arteries and arterioles	6	2
Renal failure/disease of kidneys	3	—
Diseases of the musculoskeletal system	5	1
Osteochondrodysplasias	1	—
Fractures/disorders of continuity of bone	34	5
Osteomyelitis	4	1
Other disorders of bone (for example, osteonecrosis, Paget disease)	3	—
Sum	111	15
Medication and Treatments		
Chemotherapeutics	5	1
Corticosteroids	106	9
Sex hormones	1	1
Pituitary and hypothalamic hormones and analogues	2	—
Tetracyclines	1	1
Sum	115	12

Abbreviations: CTX, cross-linking telopeptide of type I collagen; SI, stiffness index.

RESULTS

Characteristics of the analysis groups for SI and CTX

Tables 2 and 3 present the sex-specific characteristics of the SI and CTX analysis groups, stratified by 1-year age groups, respectively. The SI analysis group included children aged 2–10.9 years and consisted of 5412 boys and 5379 girls. Boys and girls had similar average SI values. Although, preschool girls tended to have slightly higher SI values compared with boys, the primary school girls had marginally lower SI values. Age-specific average body height and weight were similar in boys and girls (see Table 2).

The CTX analysis group aged 3–8.9 years consisted of 857 boys and 789 girls. Within 1-year age groups, CTX values differed by

0.01–0.07 ng ml^{-1} between both sexes. With increasing age, slightly higher mean CTX values were observed in boys and girls. Boys and girls showed similar age-specific body height and weight values (see Table 3).

Age- and sex-specific reference percentiles for SI

Tables 4 and 5 show the 1st, 3rd, 10th, 25th, 50th, 75th, 90th, 97th and 99th SI percentile values by 1-year age groups and height for girls and boys, separately. Figure 4 presents sex-specific reference curves of the 1st, 50th and 99th SI percentile by age for small, average and tall children (that is, 3rd, 50th and 97th height percentile).

In preschool children, SI percentiles showed a negative association with age and height. In primary school children, a positive association with age and height was observed. The dip in SI occurred at an older age for higher SI percentiles and was observed earlier in taller children than in smaller children. The association of SI with height was inverted around the dip. In boys, height had a smaller effect on SI after the dip compared with girls (Figure 4 and Tables 4 and 5).

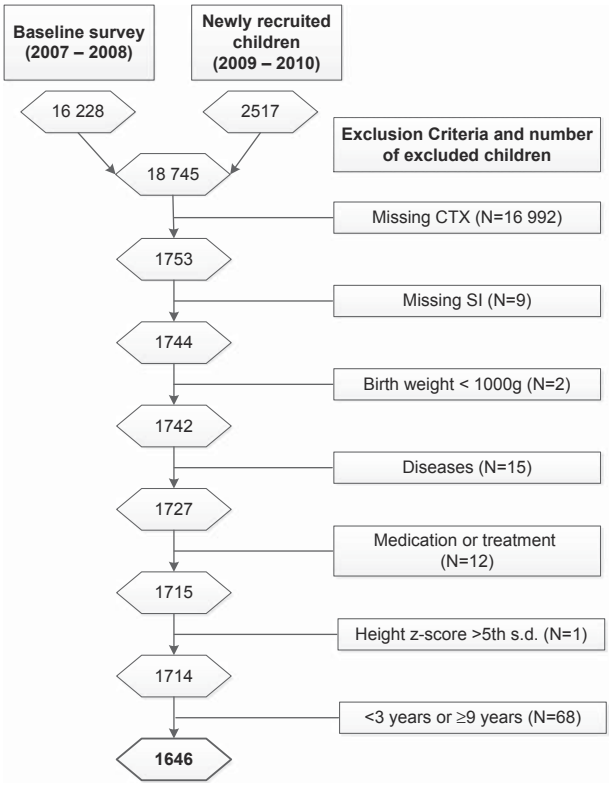

Figure 2. Number of included and excluded children per exclusion criteria for the CTX analysis group from the IDEFICS population.

Age- and sex-specific reference percentiles for CTX

Tables 6 and 7 show the 1st, 3rd, 10th, 25th, 50th, 75th, 90th, 97th and 99th CTX percentile values by 1-year age groups and height for girls and boys, separately. Figure 5 presents sex-specific reference curves of the 1st, 50th and 99th CTX percentile by age for small, average and tall children. The CTX values showed a linear-positive association with age and height. The impact of height was slightly stronger in boys than in girls.

DISCUSSION

This study provides the first sex-, age- and height-specific reference values for calcaneal SI and serum CTX for 2–10.9- and 3–8.9-year-old children, respectively. Both analysis groups are based on a population of apparently healthy European children, where standardised QUS and blood measurements were obtained.

The main observation was the negative trend in SI with increasing age and height in preschool children, which was followed by a positive trend in SI with age and height in primary school children. The negative trend of SI in preschool children may be explained by the increased height velocity in early childhood[47] where bone turnover processes may have not adequately adapted to the growth velocity in young children. Due to the lack of bone reference data in preschool children, no comparison with other data can be made. The few studies in children mostly reported data above the age of 6 years and observed a positive trend of bone parameters with increasing age.[21] Most bone reference data in children are based on DXA measurements. But it remains questionable to compare QUS with DXA owing to the two-dimensional image projection of DXA measures, and the problem that aBMD is not accounting for growth. Mathematical models were established to calculate the volumetric BMD or bone mineral apparent density, where height, bone area and bone width are considered to avoid under- or overestimation of bone status.[16,18,19,22] Considering such models, a slight decline in bone mineral apparent density of the femoral neck with increasing age has been observed in primary school children, which is in contrast to our findings.[16,19]

A comparison of our QUS reference data with other QUS reference data should be made with caution. Most of the studies used different methods for calculating reference values or applied different QUS devices (for example, CUBA, Sahara, Sunlight Omnisense) with different measures (for example, speed of sound, broadband ultrasound attenuation) on different body sites (for

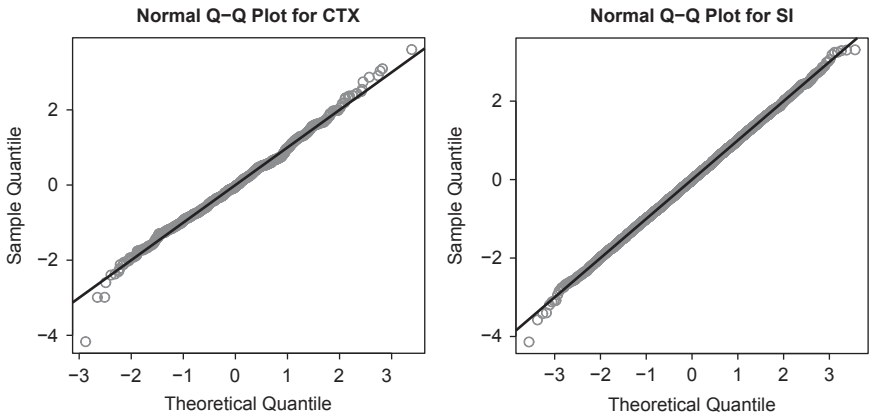

Figure 3. Q–Q plot of residuals for SI and CTX in boys.

Table 2. Sex-specific SI, height and weight of the IDEFICS SI analysis group, stratified by 1-year age groups

Age group (year)	*Girls*					*Boys*				
	N	*Age (year)* mean ± s.d.	*SI* mean ± s.d.	*Height (cm)* mean ± s.d.	*Weight (kg)* mean ± s.d.	N	*Age (year)* mean ± s.d.	*SI* mean ± s.d.	*Height (cm)* mean ± s.d.	*Weight (kg)* mean ± s.d.
2.0–2.9	102	2.7 ± 0.2	84.7 ± 10.5	93.0 ± 4.1	13.9 ± 1.8	116	2.6 ± 0.2	84.6 ± 11.9	93.9 ± 4.3	14.6 ± 1.9
3.0–3.9	476	3.5 ± 0.3	82.5 ± 15.2	100.4 ± 5.1	16.0 ± 2.5	495	3.5 ± 0.3	81.7 ± 13.7	101.0 ± 4.9	16.2 ± 2.1
4.0–4.9	593	4.5 ± 0.3	82.4 ± 17.4	106.8 ± 4.7	18.1 ± 2.8	670	4.5 ± 0.3	80.2 ± 15.4	108.1 ± 5.1	18.6 ± 3.1
5.0–5.9	628	5.5 ± 0.3	80.0 ± 17.5	113.9 ± 5.3	20.7 ± 3.7	680	5.5 ± 0.3	79.1 ± 16.0	114.7 ± 5.2	21.0 ± 3.6
6.0–6.9	768	6.5 ± 0.3	76.9 ± 14.6	120.0 ± 5.5	23.4 ± 4.7	762	6.5 ± 0.3	77.8 ± 13.8	121.3 ± 5.6	24.1 ± 4.8
7.0–7.9	1104	7.5 ± 0.3	78.8 ± 11.9	126.7 ± 5.5	27.0 ± 5.7	1078	7.5 ± 0.3	80.1 ± 11.7	127.9 ± 5.8	27.8 ± 6.0
8.0–8.9	911	8.4 ± 0.3	81.1 ± 12.0	131.7 ± 5.9	30.4 ± 6.6	836	8.4 ± 0.3	82.0 ± 11.5	132.6 ± 5.8	30.5 ± 6.9
9.0–9.9	535	9.5 ± 0.3	83.8 ± 10.6	138.0 ± 6.7	34.4 ± 8.5	473	9.5 ± 0.3	84.8 ± 11.7	139.3 ± 6.4	36.0 ± 8.6
10.0–10.9	262	10.3 ± 0.3	85.8 ± 12.6	143.9 ± 6.5	39.7 ± 9.9	302	10.3 ± 0.3	87.0 ± 11.8	142.9 ± 6.4	39.3 ± 10.2

Abbreviations: IDEFICS, Identification and prevention of Dietary- and lifestyle-induced health Effects in Children and InfantS; SI, stiffness index.

Table 3. Sex-specific CTX, height and weight of the IDEFICS CTX analysis group, stratified by 1-year age groups

Age group (year)	*Girls*					*Boys*				
	N	*Age (year)* *mean* ± s.d.	*CTX (ng ml^{-1})* *mean* ± s.d.	*Height (cm)* *mean* ± s.d.	*Weight (kg)* *mean* ± s.d.	N	*Age (year)* *mean* ± s.d.	*CTX (ng/ml)* *mean* ± s.d.	*Height (cm)* *mean* ± s.d.	*Weight (kg)* *mean* ± s.d.
3.0–3.9	109	3.5 ± 0.3	1.06 ± 0.24	101.1 ± 4.8	16.2 ± 2.9	94	3.6 ± 0.3	1.09 ± 0.26	102.1 ± 4.9	16.7 ± 2.5
4.0–4.9	117	4.4 ± 0.3	1.19 ± 0.28	107.2 ± 4.2	18.2 ± 2.6	152	4.5 ± 0.3	1.13 ± 0.28	108.2 ± 4.7	18.5 ± 2.6
5.0–5.9	103	5.4 ± 0.3	1.23 ± 0.30	113.9 ± 5.2	20.9 ± 3.8	111	5.4 ± 0.3	1.17 ± 0.25	114.7 ± 4.9	20.5 ± 2.8
6.0–6.9	129	6.5 ± 0.3	1.20 ± 0.27	120.1 ± 5.0	22.8 ± 5.0	154	6.5 ± 0.3	1.21 ± 0.25	122.0 ± 5.2	24.0 ± 4.6
7.0–7.9	186	7.5 ± 0.3	1.24 ± 0.27	126.2 ± 5.3	26.2 ± 5.2	198	7.5 ± 0.3	1.22 ± 0.26	127.4 ± 6.0	27.3 ± 6.0
8.0–8.9	145	8.4 ± 0.3	1.25 ± 0.29	130.9 ± 5.6	29.0 ± 6.2	148	8.4 ± 0.3	1.25 ± 0.25	131.6 ± 6.0	28.8 ± 6.2

Abbreviations: CTX, cross-linking telopeptide of type I collagen; IDEFICS, Identification and prevention of Dietary- and lifestyle-induced health Effects in Children and InfantS; SI, stiffness index.

example, radius, phalanx) in different ethnicities.[20,48–54] The few studies that assessed calcaneal QUS in prepubertal children aged 6–10 years showed a progressive increase of broadband ultrasound attenuation and a small or no increase of speed of sound with age.[48,50–52] Only two studies established SI reference or normative data in children, adolescents and young adults in the age range from 6 to 21 years. Compared with prepubertal ages, both studies observed a stronger trend in SI with age around the age of 12 years in girls and 14 years in boys.[20,54] This increase during puberty was also found in studies considering other QUS or DXA bone parameters, indicating that the growth spurt started earlier in girls than boys.[14,21,50,51] In the present study, no such spurt in SI was observed until the age of 10 years. However, our data showed a positive trend of height in tall 10-year old girls, which may indicate the earlier start of maturation in girls than in boys.

The few published references and normative data on SI [20,54] and CTX [15,55] are not informative regarding age distribution, especially not of the more extreme percentiles, which are of interest for clinical practice. We suggest that the lower the SI percentiles, the more important the meaning of the increase or decrease in SI. In other words, a difference of five SI units between age groups would be more relevant for bone diagnostic at the 3rd SI percentile than at the 50th percentile. For example, an increase of five units in a 5.5-year-old girl with an SI in the 3rd percentile would result in an improvement of SI from the 3rd to the 10th percentile (see Table 4). The same applies to CTX.

Considering the present SI reference values, the 3rd age-specific percentile in 5.5–10.9-year-old boys (range: 55.7–69.4) and girls (range: 54.2–69.1) may be considered as a warning threshold for low SI and reduced skeletal development. The pathological meaning of these values still needs to be evaluated. Only one study investigated SI values in children with bone disorders: Jaworski *et al.*[27] observed a mean SI of 50.6 ± 13.7 in eighteen 6- to 13-year old children having osteopenia. According to these findings, our reference values for 5.5–9.5-year- old girls in the 1st age-specific SI percentile (range: 49.9–64.9) are close to this pathological value for osteopenia. The observed range of 15 SI units of the 1st percentile between the age of 5 and 10 years supports the need for age-specific cut-off values of SI, for example, for predicting osteopenia in childhood.

CTX is mainly used as a bone resorption marker in post-menopausal women. Compared with adults, elevated CTX concentrations of children can be explained by the high skeletal growth velocity and rapid bone turnover.[56,57] Only one study reported reference values for the 5th, 50th and 95th percentiles established from a smaller sample of 572 Caucasian children. Although a different assay for analysing CTX was used in this study, it showed similar CTX values.[15] Other studies reported different CTX values in prepubertal children. For example, Crofton *et al.*[55] observed a 95% reference interval (median of 0.352 ng ml^{-1}) be1tween 0.146 and 0.818 ng ml^{-1} in a small study sample of 124 Caucasian boys and girls aged 1–9 years. These concentrations are below the reference range of our 3–8-year-old CTX study sample, which varied between 0.20 and 2.03 ng ml^{-1}. In contrast, Ambroszkiewiki *et al.*[58] reported different means of CTX in 100 vegetarian (1.697 ± 0.653 ng ml^{-1}) and omnivorous children (1.99 ± 0.30 ng ml^{-1}) aged 2–10 years, which compares with a lower mean of CTX in our study group (1.19 ± 0.27 ng ml^{-1}). Crofton *et al.* and Ambroszkiewiki *et al.* used a different assay for analysing CTX which may be one reason for the differences.

To present reference values that are not affected by disturbed skeletal development due to very low birth weight, diseases or

Table 4. SI percentile values (P) for calcaneal QUS by 1-year age groups and age-specific height percentiles (3rd, 10th, 25th, 50th, 75th, 90th and 97th) in girls

Age (year)	*Height (cm)*	*Stiffness index percentiles—girls*								
		P1	*P3*	*P10*	*P25*	*P50*	*P75*	*P90*	*P97*	*P99*
2.5	85	60.2	65.8	72.9	79.8	87.0	93.9	99.9	105.7	109.8
2.5	87	58.5	64.2	71.5	78.6	86.3	93.6	100.0	106.2	110.7
2.5	89	57.0	62.7	70.2	77.5	85.5	93.3	100.2	106.8	111.7
2.5	92	54.7	60.5	68.1	75.8	84.4	92.9	100.5	108.0	113.6
2.5	94	53.4	59.1	66.8	74.7	83.6	92.6	100.8	109.0	115.2
2.5	97	51.7	57.2	64.8	73.0	82.4	92.2	101.4	110.7	117.9
2.5	100	50.3	55.5	63.0	71.2	81.0	91.6	101.9	112.7	121.1
3.5	92	55.2	60.9	68.6	76.4	85.0	93.6	101.3	108.9	114.5
3.5	95	53.2	58.9	66.6	74.7	83.9	93.2	101.8	110.4	116.9
3.5	97	52.1	57.6	65.4	73.5	83.0	92.9	102.2	111.6	118.9
3.5	100	50.7	56.0	63.5	71.8	81.7	92.4	102.7	113.6	122.1
3.5	102	50.0	55.0	62.4	70.6	80.7	91.9	103.0	114.8	124.4
3.5	105	49.2	53.9	60.9	68.9	79.1	90.9	102.9	116.5	127.7
3.5	108	49.1	53.3	59.8	67.5	77.5	89.5	102.3	117.4	130.5
4.5	98	52.0	57.5	65.3	73.5	83.3	93.5	103.2	113.2	120.9
4.5	101	50.7	55.9	63.4	71.8	81.9	92.9	103.7	115.2	124.2
4.5	104	49.8	54.7	61.9	70.1	80.3	92.0	103.9	116.9	127.7
4.5	107	49.5	53.9	60.6	68.5	78.7	90.7	103.5	118.2	130.8
4.5	110	49.8	53.8	59.9	67.3	77.1	89.1	102.4	118.5	133.0
4.5	113	50.6	54.2	59.8	66.5	75.7	87.2	100.5	117.4	133.4
4.5	116	51.7	55.0	60.1	66.2	74.7	85.5	98.1	114.6	130.8
5.5	104	50.2	55.1	62.4	70.6	81.0	92.8	104.7	117.9	128.7
5.5	108	49.9	54.2	60.9	68.6	78.8	91.0	104.1	119.4	132.8
5.5	111	50.4	54.3	60.4	67.6	77.3	89.3	102.7	119.3	134.5
5.5	114	51.4	54.9	60.4	67.0	76.0	87.4	100.6	117.7	134.0
5.5	117	52.6	55.8	60.8	66.8	75.1	85.7	98.1	114.3	130.3
5.5	120	53.8	56.9	61.5	67.1	74.8	84.4	95.5	110.0	124.0
5.5	123	55.2	58.1	62.6	67.8	74.9	83.7	93.6	106.1	117.8
6.5	110	50.6	54.7	61.0	68.5	78.4	90.6	104.2	120.5	135.3
6.5	113	51.5	55.2	60.8	67.7	77.0	88.8	102.3	119.4	135.7
6.5	117	53.1	56.3	61.3	67.4	75.8	86.4	98.9	115.3	131.5
6.5	120	54.3	57.4	62.1	67.7	75.4	85.1	96.4	110.9	125.1
6.5	123	55.7	58.6	63.1	68.5	75.6	84.4	94.4	107.0	118.9
6.5	127	57.7	60.5	64.8	69.8	76.4	84.4	93.1	103.8	113.5
6.5	130	59.1	61.9	66.1	71.0	77.4	85.0	93.2	103.0	111.6
7.5	115	52.7	56.2	61.5	68.0	77.0	88.3	101.6	118.8	135.5
7.5	119	54.4	57.5	62.3	68.2	76.1	86.3	98.0	113.4	128.5
7.5	123	56.2	59.2	63.7	69.1	76.2	85.1	95.2	107.9	119.9
7.5	126	57.7	60.6	64.9	70.1	76.8	85.0	94.1	105.3	115.5
7.5	130	59.6	62.4	66.7	71.6	78.1	85.7	94.0	103.8	112.6
7.5	133	60.8	63.6	67.9	72.8	79.2	86.6	94.6	104.0	112.1
7.5	137	62.0	64.9	69.3	74.3	80.6	88.0	95.9	104.9	112.6
8.5	120	55.3	58.4	63.2	68.9	76.7	86.6	98.1	112.9	127.3
8.5	124	57.2	60.1	64.6	70.0	77.0	85.8	95.6	107.8	119.2
8.5	128	59.2	62.0	66.4	71.4	78.0	86.0	94.7	105.2	114.6
8.5	132	60.9	63.8	68.1	73.1	79.5	87.0	95.2	104.7	113.1
8.5	136	62.2	65.2	69.5	74.5	80.9	88.4	96.3	105.5	113.4
8.5	139	62.9	65.9	70.4	75.5	82.0	89.5	97.3	106.4	114.1
8.5	143	63.4	66.6	71.2	76.5	83.3	91.0	99.0	108.1	115.8
9.5	125	58.2	61.1	65.6	70.9	77.9	86.5	96.0	107.8	118.8
9.5	130	60.6	63.5	67.8	72.9	79.4	87.2	95.6	105.6	114.5
9.5	134	62.1	65.1	69.4	74.4	80.9	88.4	96.5	105.9	114.0
9.5	138	63.2	66.2	70.7	75.8	82.3	89.9	97.8	106.9	114.7
9.5	142	63.8	67.0	71.6	76.9	83.6	91.3	99.3	108.5	116.2
9.5	145	64.0	67.3	72.2	77.6	84.6	92.5	100.7	110.0	117.8
9.5	149	64.0	67.5	72.7	78.5	85.8	94.1	102.7	112.2	120.2
10.5	130	61.1	64.0	68.4	73.5	80.1	87.9	96.4	106.5	115.5
10.5	135	63.0	65.9	70.3	75.4	81.9	89.5	97.6	107.0	115.1
10.5	140	64.1	67.2	71.8	77.0	83.6	91.3	99.3	108.5	116.3
10.5	144	64.5	67.7	72.6	78.0	84.9	92.8	101.0	110.3	118.1
10.5	148	64.6	68.0	73.1	78.9	86.2	94.5	103.0	112.5	120.5
10.5	152	64.4	68.1	73.6	79.7	87.4	96.2	105.0	114.9	123.0
10.5	156	64.0	68.0	74.0	80.6	88.7	97.9	107.1	117.2	125.5

Abbreviations: QUS, quantitative ultrasound; SI, stiffness index.

Table 5. SI percentile values (P) for calcaneal quantitative ultrasound by 1-year age groups and age-specific height percentiles (3rd, 10th, 25th, 50th, 75th, 90th and 97th) in boys

Age (year)	*Height (cm)*	*Stiffness index percentiles—boys*								
		P1	*P3*	*P10*	*P25*	*P50*	*P75*	*P90*	*P97*	*P99*
2.5	85	63.0	68.6	75.7	82.4	89.4	95.9	101.6	107.0	110.8
2.5	88	60.4	66.0	73.2	80.1	87.5	94.5	100.7	106.6	110.8
2.5	91	57.9	63.4	70.6	77.8	85.6	93.2	99.9	106.4	111.2
2.5	93	56.3	61.7	69.0	76.3	84.3	92.3	99.4	106.4	111.6
2.5	96	54.1	59.3	66.5	74.0	82.4	90.9	98.7	106.6	112.4
2.5	98	52.8	57.9	65.0	72.5	81.1	90.0	98.3	106.8	113.2
2.5	101	51.1	55.9	62.8	70.3	79.2	88.7	97.8	107.3	114.7
3.5	92	58.1	63.6	71.0	78.4	86.4	94.3	101.4	108.2	113.3
3.5	95	55.8	61.2	68.5	76.1	84.5	93.0	100.7	108.4	114.1
3.5	98	53.7	58.9	66.2	73.8	82.6	91.7	100.2	108.7	115.3
3.5	101	52.1	57.0	64.0	71.6	80.7	90.4	99.6	109.3	116.9
3.5	103	51.2	55.9	62.7	70.3	79.4	89.5	99.3	109.8	118.1
3.5	106	50.2	54.6	61.1	68.4	77.6	88.0	98.6	110.4	120.0
3.5	109	49.8	53.8	59.8	66.8	75.8	86.5	97.7	110.7	121.8
4.5	99	54.1	59.3	66.6	74.4	83.4	92.9	101.8	110.9	117.9
4.5	102	52.5	57.5	64.5	72.3	81.5	91.6	101.3	111.6	119.6
4.5	105	51.5	56.0	62.7	70.3	79.7	90.2	100.8	112.3	121.6
4.5	108	50.9	55.1	61.4	68.6	77.9	88.7	100	112.8	123.6
4.5	111	50.7	54.6	60.4	67.2	76.2	87.1	98.7	112.6	124.8
4.5	114	51.0	54.5	59.9	66.3	74.9	85.3	96.9	111.1	124.0
4.5	117	51.7	55.0	60.0	65.9	73.9	83.7	94.7	108.4	121.1
5.5	105	52.4	57.1	63.9	71.6	81.2	91.9	102.6	114.4	123.9
5.5	108	51.8	56.1	62.5	69.9	79.4	90.4	101.9	115.0	126.0
5.5	111	51.7	55.6	61.6	68.6	77.7	88.8	100.7	114.8	127.2
5.5	115	52.2	55.7	61.1	67.5	76.0	86.4	98.1	112.5	125.7
5.5	118	53.1	56.3	61.3	67.2	75.1	84.9	95.9	109.6	122.2
5.5	121	54.1	57.2	61.8	67.4	74.7	83.8	93.9	106.4	118.0
5.5	125	55.7	58.6	63.0	68.1	74.9	83.1	92.2	103.2	113.3
6.5	111	52.7	56.7	62.8	69.9	79.2	90.5	102.6	117.0	129.7
6.5	115	53.2	56.8	62.3	68.8	77.5	88.1	100.1	114.7	128.2
6.5	118	54.1	57.4	62.5	68.5	76.6	86.6	97.8	111.7	124.7
6.5	121	55.2	58.3	63.1	68.7	76.2	85.4	95.8	108.5	120.3
6.5	125	56.8	59.7	64.2	69.5	76.4	84.8	94.0	105.3	115.5
6.5	128	57.9	60.8	65.2	70.2	76.9	84.8	93.5	103.9	113.3
6.5	132	59.2	62.1	66.3	71.3	77.7	85.3	93.5	103.2	111.8
7.5	117	54.8	58.3	63.6	69.9	78.3	88.8	100.5	115.0	128.4
7.5	120	55.9	59.1	64.1	70.0	77.8	87.4	98.3	111.7	124.2
7.5	124	57.5	60.5	65.2	70.6	77.8	86.5	96.2	108.0	118.8
7.5	127	58.7	61.6	66.1	71.3	78.2	86.4	95.4	106.3	116.1
7.5	131	60.1	63.0	67.3	72.4	79.0	86.8	95.2	105.3	114.2
7.5	134	60.8	63.7	68.1	73.1	79.6	87.3	95.4	105.1	113.5
7.5	138	61.4	64.4	68.9	74.0	80.4	88.0	96.0	105.2	113.1
8.5	122	57.8	61.0	65.8	71.6	79.2	88.5	98.9	111.8	123.6
8.5	126	59.4	62.5	67.0	72.4	79.5	88.0	97.4	108.8	119.1
8.5	129	60.6	63.5	68.0	73.2	80.0	88.2	97.1	107.7	117.2
8.5	133	61.7	64.7	69.1	74.3	80.9	88.7	97.1	107.1	115.8
8.5	137	62.4	65.5	70.0	75.1	81.7	89.5	97.6	107.1	115.2
8.5	140	62.7	65.8	70.4	75.7	82.3	90.0	98.1	107.3	115.1
8.5	144	62.8	66.1	70.9	76.3	83.1	90.8	98.7	107.7	115.1
9.5	127	60.9	64.0	68.6	74.1	81.1	89.7	99.1	110.4	120.6
9.5	131	62.3	65.4	69.9	75.1	82.0	90.1	98.8	109.3	118.5
9.5	134	63.1	66.1	70.6	75.9	82.6	90.5	99.0	109.0	117.7
9.5	138	63.7	66.8	71.4	76.7	83.4	91.3	99.5	109.1	117.2
9.5	142	64.0	67.2	72.0	77.4	84.2	92.0	100.2	109.4	117.2
9.5	146	63.9	67.3	72.3	77.9	84.9	92.8	100.8	109.8	117.2
9.5	150	63.6	67.2	72.5	78.3	85.5	93.4	101.3	110.0	116.9
10.5	132	63.7	66.8	71.4	76.8	83.7	91.9	100.7	111.1	120.3
10.5	135	64.4	67.5	72.1	77.5	84.3	92.3	100.9	110.9	119.6
10.5	139	64.9	68.1	72.9	78.3	85.1	93.1	101.5	111.1	119.3
10.5	143	65.1	68.4	73.3	78.9	85.9	93.9	102.1	111.4	119.3
10.5	148	64.9	68.4	73.7	79.5	86.7	94.7	102.9	111.8	119.1
10.5	152	64.5	68.3	73.8	79.9	87.2	95.3	103.3	111.9	118.8
10.5	155	64.1	68.1	73.9	80.1	87.6	95.8	103.7	112.0	118.6

Abbreviation: SI, stiffness index.

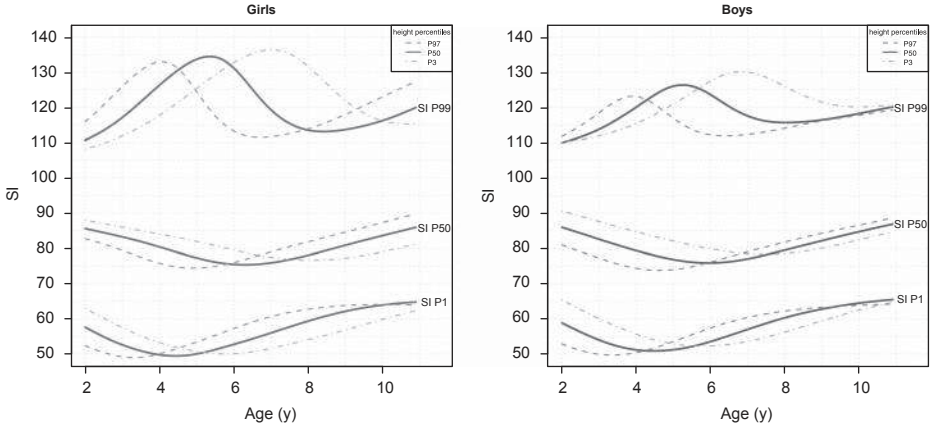

Figure 4. SI reference curves of the 1st, 50th and 99th percentiles (P) for girls and boys considering the 3rd, 50th and 97th age- and sex-specific height percentiles.

Table 6. CTX percentile values (P) by 1-year age groups and age-specific height percentiles (3rd, 10th, 25th, 50th, 75th, 90th and 97th) in girls

Age (year)	*Height (cm)*	*CTX percentiles—girls*								
		P1	*P3*	*P10*	*P25*	*P50*	*P75*	*P90*	*P97*	*P99*
3.5	92	0.42	0.54	0.70	0.86	1.05	1.23	1.39	1.55	1.67
3.5	95	0.44	0.56	0.72	0.89	1.07	1.25	1.41	1.58	1.70
3.5	97	0.46	0.58	0.74	0.90	1.09	1.27	1.43	1.59	1.71
3.5	100	0.48	0.60	0.76	0.93	1.11	1.29	1.46	1.62	1.74
3.5	102	0.50	0.62	0.78	0.94	1.13	1.31	1.47	1.63	1.75
3.5	105	0.52	0.64	0.81	0.97	1.15	1.33	1.50	1.66	1.78
3.5	108	0.55	0.67	0.83	0.99	1.17	1.36	1.52	1.68	1.80
4.5	98	0.45	0.57	0.73	0.90	1.08	1.26	1.42	1.59	1.71
4.5	101	0.48	0.60	0.76	0.92	1.10	1.28	1.45	1.61	1.73
4.5	104	0.50	0.62	0.78	0.95	1.13	1.31	1.47	1.63	1.75
4.5	107	0.52	0.64	0.81	0.97	1.15	1.33	1.50	1.66	1.78
4.5	110	0.55	0.67	0.83	0.99	1.18	1.36	1.52	1.68	1.80
4.5	113	0.57	0.69	0.85	1.02	1.20	1.38	1.55	1.71	1.83
4.5	116	0.60	0.72	0.88	1.04	1.22	1.41	1.57	1.73	1.85
5.5	104	0.49	0.61	0.77	0.93	1.11	1.29	1.46	1.62	1.74
5.5	108	0.52	0.64	0.80	0.96	1.14	1.33	1.49	1.65	1.77
5.5	111	0.54	0.66	0.82	0.99	1.17	1.35	1.51	1.68	1.80
5.5	114	0.57	0.69	0.85	1.01	1.19	1.37	1.54	1.70	1.82
5.5	117	0.59	0.71	0.87	1.04	1.22	1.40	1.56	1.72	1.84
5.5	120	0.61	0.73	0.90	1.06	1.24	1.42	1.59	1.75	1.87
5.5	123	0.64	0.76	0.92	1.08	1.27	1.45	1.61	1.77	1.89
6.5	110	0.52	0.64	0.80	0.96	1.15	1.33	1.49	1.65	1.77
6.5	113	0.54	0.66	0.82	0.99	1.17	1.35	1.51	1.68	1.80
6.5	117	0.58	0.70	0.86	1.02	1.20	1.38	1.55	1.71	1.83
6.5	120	0.60	0.72	0.88	1.04	1.23	1.41	1.57	1.73	1.85
6.5	123	0.62	0.74	0.91	1.07	1.25	1.43	1.60	1.76	1.88
6.5	127	0.66	0.78	0.94	1.10	1.28	1.46	1.63	1.79	1.91
6.5	130	0.68	0.80	0.96	1.13	1.31	1.49	1.65	1.81	1.93
7.5	115	0.54	0.66	0.83	0.99	1.17	1.35	1.52	1.68	1.80
7.5	119	0.58	0.70	0.86	1.02	1.20	1.38	1.55	1.71	1.83
7.5	123	0.61	0.73	0.89	1.05	1.24	1.42	1.58	1.74	1.86
7.5	126	0.63	0.75	0.91	1.08	1.26	1.44	1.60	1.77	1.89
7.5	130	0.67	0.79	0.95	1.11	1.29	1.47	1.64	1.80	1.92
7.5	133	0.69	0.81	0.97	1.13	1.32	1.50	1.66	1.82	1.94
7.5	137	0.72	0.84	1.00	1.17	1.35	1.53	1.69	1.85	1.98
8.5	120	0.57	0.69	0.85	1.01	1.20	1.38	1.54	1.70	1.82
8.5	124	0.60	0.72	0.88	1.05	1.23	1.41	1.57	1.73	1.85
8.5	128	0.63	0.75	0.92	1.08	1.26	1.44	1.61	1.77	1.89
8.5	132	0.67	0.79	0.95	1.11	1.29	1.47	1.64	1.80	1.92
8.5	136	0.70	0.82	0.98	1.14	1.33	1.51	1.67	1.83	1.95
8.5	139	0.72	0.84	1.00	1.17	1.35	1.53	1.69	1.86	1.98
8.5	143	0.75	0.87	1.04	1.20	1.38	1.56	1.73	1.89	2.01

Abbreviation: CTX, cross-linking telopeptide of type I collagen.

Table 7. Percentile values (P) of CTX by 1-year age groups and age-specific height percentiles (3rd, 10th, 25th, 50th, 75th, 90th and 97th) in boys

Age (year)	*Height (cm)*	*CTX percentiles—boys*								
		P1	*P3*	*P10*	*P25*	*P50*	*P75*	*P90*	*P97*	*P99*
3.5	92	0.39	0.51	0.66	0.81	0.98	1.15	1.31	1.46	1.57
3.5	95	0.42	0.54	0.69	0.84	1.02	1.19	1.34	1.49	1.61
3.5	98	0.46	0.57	0.72	0.88	1.05	1.22	1.37	1.52	1.64
3.5	101	0.49	0.60	0.75	0.91	1.08	1.25	1.40	1.56	1.67
3.5	103	0.51	0.62	0.77	0.93	1.10	1.27	1.43	1.58	1.69
3.5	106	0.54	0.65	0.81	0.96	1.13	1.30	1.46	1.61	1.72
3.5	109	0.57	0.69	0.84	0.99	1.16	1.34	1.49	1.64	1.75
4.5	99	0.44	0.55	0.70	0.85	1.03	1.20	1.35	1.50	1.62
4.5	102	0.47	0.58	0.73	0.89	1.06	1.23	1.38	1.54	1.65
4.5	105	0.50	0.61	0.76	0.92	1.09	1.26	1.42	1.57	1.68
4.5	108	0.53	0.64	0.80	0.95	1.12	1.29	1.45	1.60	1.71
4.5	111	0.56	0.68	0.83	0.98	1.15	1.32	1.48	1.63	1.74
4.5	114	0.59	0.71	0.86	1.01	1.19	1.36	1.51	1.66	1.78
4.5	117	0.63	0.74	0.89	1.05	1.22	1.39	1.54	1.70	1.81
5.5	105	0.47	0.58	0.73	0.89	1.06	1.23	1.38	1.54	1.65
5.5	108	0.50	0.61	0.76	0.92	1.09	1.26	1.42	1.57	1.68
5.5	111	0.53	0.64	0.80	0.95	1.12	1.29	1.45	1.60	1.71
5.5	115	0.57	0.69	0.84	0.99	1.16	1.34	1.49	1.64	1.76
5.5	118	0.61	0.72	0.87	1.03	1.20	1.37	1.52	1.67	1.79
5.5	121	0.64	0.75	0.90	1.06	1.23	1.40	1.55	1.71	1.82
5.5	125	0.68	0.79	0.95	1.10	1.27	1.44	1.60	1.75	1.86
6.5	111	0.50	0.61	0.76	0.92	1.09	1.26	1.42	1.57	1.68
6.5	115	0.54	0.66	0.81	0.96	1.13	1.30	1.46	1.61	1.72
6.5	118	0.57	0.69	0.84	0.99	1.16	1.34	1.49	1.64	1.76
6.5	121	0.61	0.72	0.87	1.03	1.20	1.37	1.52	1.67	1.79
6.5	125	0.65	0.76	0.91	1.07	1.24	1.41	1.56	1.72	1.83
6.5	128	0.68	0.79	0.95	1.10	1.27	1.44	1.60	1.75	1.86
6.5	132	0.72	0.84	0.99	1.14	1.31	1.48	1.64	1.79	1.90
7.5	117	0.53	0.64	0.80	0.95	1.12	1.29	1.45	1.60	1.71
7.5	120	0.56	0.68	0.83	0.98	1.15	1.33	1.48	1.63	1.75
7.5	124	0.61	0.72	0.87	1.03	1.20	1.37	1.52	1.67	1.79
7.5	127	0.64	0.75	0.90	1.06	1.23	1.40	1.55	1.71	1.82
7.5	131	0.68	0.79	0.95	1.10	1.27	1.44	1.60	1.75	1.86
7.5	134	0.71	0.83	0.98	1.13	1.30	1.47	1.63	1.78	1.89
7.5	138	0.75	0.87	1.02	1.17	1.35	1.52	1.67	1.82	1.94
8.5	122	0.55	0.67	0.82	0.97	1.14	1.32	1.47	1.62	1.73
8.5	126	0.60	0.71	0.86	1.02	1.19	1.36	1.51	1.66	1.78
8.5	129	0.63	0.74	0.89	1.05	1.22	1.39	1.54	1.70	1.81
8.5	133	0.67	0.78	0.94	1.09	1.26	1.43	1.59	1.74	1.85
8.5	137	0.71	0.83	0.98	1.13	1.30	1.47	1.63	1.78	1.89
8.5	140	0.74	0.86	1.01	1.16	1.34	1.51	1.66	1.81	1.93
8.5	144	0.79	0.90	1.05	1.21	1.38	1.55	1.70	1.86	1.97

Abbreviation: CTX, cross-linking telopeptide of type I collagen.

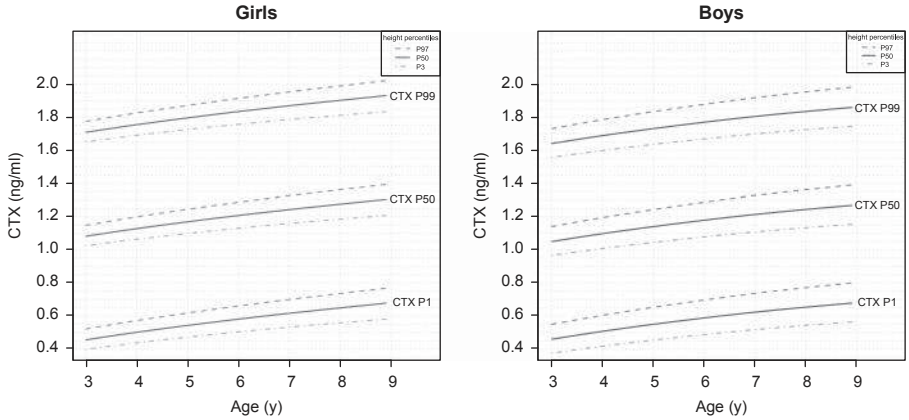

Figure 5. CTX reference curves of the 1st, 50th and 99th percentiles (P) for girls and boys considering the 3rd, 50th and 97th age- and sex-specific height percentile.

medical treatments that directly affect bone metabolism, children with these conditions were excluded. Fat mass may also have a pathophysiological effect on bone metabolism.[2–4] This is further supported by Eliakim *et al.*[59] who showed reduced QUS measures in obese compared to non-obese children. Furthermore, a pathophysiological relationship of excessive thinness on bone health has been reported.[60] We performed sensitivity analyses to test the robustness of the SI and CTX reference percentiles comparing one model excluding underweight (stage 3 according the International Obesity Task Force criteria)[42] and obese children with our main model in which these children were included. SI and CTX values obtained from both models had only minor deviations (Supplementary Figures A and B). Therefore, we retained underweight and obese children in the analysis group. Reference curves excluding underweight and obese children are provided in (Supplementary Figures C and D).

A limitation of this study is the cross-sectional design. Longitudinal data of individuals give more accurate reference data. Furthermore, the lack of studies investigating long-term sequels of low SI or inadequate CTX concentrations limits our ability to propose risk-based cut-offs. We investigated serum CTX as an indicator of bone metabolism. Several studies highlighted the importance of considering more than one bone metabolic marker, that is, at least one bone formation and resorption marker each, for assessing reduced bone turnover or a decreased skeletal development.[15]

The use of an unselected, large and heterogeneous sample of apparently healthy European children with different living conditions and providing data on SI and CTX using standardised QUS, blood and anthropometric measures is a strength of the current study. Furthermore, the large examination programme of the IDEFICS study allowed the exclusion of children suffering from conditions, diseases or that received medical treatments possibly affecting bone metabolism. As an additional strength, this study is the first applying an improved statistical method to derive SI and CTX reference percentiles by modelling age and height simultaneously.

CONCLUSION

This is the first paper providing sex-, age- and height-specific reference values of SI and serum CTX for European children aged 2–10.9 years and 3–8.9 years, respectively, based on a large and apparently healthy cohort. These reference values lay the ground to evaluate bone growth and metabolism in children in epidemiological and clinical studies. They are also of immediate use for clinical practice in children receiving treatments that possibly affect bone health as well as to monitor their skeletal development. However, to apply the present reference values, the use of different QUS brands (e.g. CUBA, Sahara, Sunlight) or CTX assays should be considered. These reference values will be complemented by data of adolescents participating in the follow-up survey of the IDEFICS study (I.Family).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Appendix E

Understanding the Links among Neuromedin U gene, Beta2-adrenoceptor Gene and Bone Health: An Observational Study in European Children

Gianfagna F, Cugino D, Ahrens W, Bailey ME, Bammann K, **Herrmann D**, Koni AC, Kourides Y, Mårild S, Molnár D, Moreno LA, Pitsiladis YP, Russo P, Siani A, Sieri S, Sioen I, Veidebaum T, Iacoviello L, and on behalf of the IDEFICS Consortium

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Understanding the Links among *neuromedin U* Gene, *beta2-adrenoceptor* Gene and Bone Health: An Observational Study in European Children

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Abstract

Neuromedin U, encoded by the *NMU* gene, is a hypothalamic neuropeptide that regulates both energy metabolism and bone mass. The beta-2 adrenergic receptor, encoded by the *ADRB2* gene, mediates several effects of catecholamine hormones and neurotransmitters in bone. We investigated whether *NMU* single nucleotide polymorphisms (SNPs) and haplotypes, as well as functional *ADRB2* SNPs, are associated with bone stiffness in children from the IDEFICS cohort, also evaluating whether *NMU* and *ADRB2* interact to affect this trait. A sample of 2,274 subjects (52.5% boys, age 6.2±1.8 years) from eight European countries, having data on calcaneus bone stiffness index (SI, mean of both feet) and genotyping (*NMU* gene: rs6827359, rs12500837, rs9999653; *ADRB2* gene: rs1042713, rs1042714), was studied. After false discovery rate adjustment, SI was significantly associated with all *NMU* SNPs. rs6827359 CC homozygotes showed the strongest association (recessive model, $\Delta = -1.8$, $p = 0.006$). Among the five retrieved haplotypes with frequencies higher than 1% (range 2.0–43.9%), the CCT haplotype (frequency = 39.7%) was associated with lower SI values (dominant model, $\Delta = -1.0$, $p = 0.04$) as compared to the most prevalent haplotype. A non-significant decrease in SI was observed in in *ADRB2* rs1042713 GG homozygotes, while subjects carrying SI-lowering genotypes at both SNPs (frequency = 8.4%) showed much lower SI than non-carriers ($\Delta = -3.9$, $p < 0.0001$; p for interaction = 0.025). The association was more evident in preschool girls, in whom SI showed a curvilinear trend across ages. In subgroup analyses, rs9999653 CC *NMU* or both GG *ADRB2* genotypes were associated with either lower serum calcium or β-CrossLaps levels ($p = 0.01$). This study in European children shows, for the first time in humans, a role for *NMU* gene through interaction with *ADRB2* gene in bone strength regulation, more evident in preschool girls.

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Introduction

Bone development is a key processes characterizing growth during childhood and adolescence [1]. Understanding this process is of crucial importance for planning strategies to prevent or treat pediatric bone disorders, as well as osteoporosis later in life [2]. While it is well known that bone homeostasis is determined by the cross-talk between osteoblasts and osteoclasts, the complexity of the regulatory influences on these cells is continuously expanding [3].

Neuromedin U (NMU) is a hypothalamic neuropeptide that regulates various metabolic functions including energy homeostasis and glycemic control [4]. Recently, Sato et al. [5] showed that *NMU* double-null mice have increased bone mass, demonstrating

also an interactive effect between single allele deletions of *NMU* and *beta-2-adrenergic receptor* (*ADRB2*). Thus, NMU is involved also in bone formation, acting as a central mediator of the effect of leptin, lead by sympathetic nervous system (SNS), on osteoblast ADRB2, which regulates cell proliferation [6,7]. No candidate gene studies have been published on humans focusing on *NMU* and bone health. Moreover, the polymorphisms most significantly associated with bone health reported by genome-wide association studies (GWAS) are located in DNA regions being far from *NMU* [8]. However, GWAS mainly focus on SNPs with large effect and did not investigate all polymorphisms in all genetic models, as well as did not consider interactions among SNPs [9].

To provide more in depth knowledge on bone health in young children, this study investigated a large sample of European children of the IDEFICS study [10]. The project aimed at identifying and preventing dietary- and lifestyle-related disorders in children and infants, mainly focusing on overweight and obesity as well as on bone health disorders, also in conjunction with overweight [11], as it shares part of its risk factor profile. In the present study we investigated the association between bone stiffness and two candidate genes, *NMU* and *ADRB2*, focusing on gene-gene interactions. Moreover, we investigated whether, in subjects carrying risk alleles, a bone loss is more evident at specific ages during childhood and whether the loss interests bone mass or microarchitecture.

Methods

Ethics Statement

The study was conducted according to the standards of the Declaration of Helsinki. All applicable institutional and governmental regulations pertaining to the ethical use of human volunteers were followed during this research. Approval by the appropriate ethical committees was obtained by each of the eight centers engaged in the fieldwork (Belgium: Ethics Committee, University Hospital, Gent; Cyprus: Cyprus National Bioethics Committee; Estonia: Tallinn Medical Research Ethics Committee; Germany: Ethics Committee, University of Bremen; Hungary: Egészségügyi Tudományos Tanács, Pécs; Italy: Comitato Etico, ASL Avellino; Spain: Comité Ético de Investigación, Clínica de Aragón - CEICA; Sweden: Regional Ethics Review Board, University of Gothenburg). Both the children and their parents gave their oral (children) and written (parents) informed consent for examinations, collection of samples, subsequent analysis and storage of personal data and collected samples.

Study Population

IDEFICS is a large European multi-center study on childhood obesity [10,12]. A cohort of 16,224 children aged 2–9 years has been recruited in a population-based survey between September 2007 and May 2008 (T0), in eight European countries (Belgium, Cyprus, Estonia, Germany, Hungary, Italy, Spain and Sweden) using standardized procedures. A community-oriented intervention program for primary prevention of obesity was implemented [13]. Children were allocated to either control or intervention group and were followed up for two years (T1, 2009–2010).

DNA was extracted from a subgroup of 4,678 samples randomly selected from the total study population, stratified by country [14,15]. All children with complete data on age, sex, parental questionnaire, height, weight, hip and waist circumferences, birthplace and language spoken at home as well as with provided saliva samples were included in the present analysis. Although no formal enquiry about ethnicity was made, two questions in the parental questionnaire provided information about ethnicity. "Place of birth of both parents" and "language habitually spoken at home" were used to select only children of European descent. DNA was successfully extracted in all cases, however, after the exclusion of samples with improbable DNA yields or not correctly genotyped, 4,641 children had at least one SNP successfully genotyped in the *NMU* (n = 4529) or *ADRB2* (n = 4566) gene. During the IDEFICS baseline survey, calcaneal quantitative ultrasound sonometry (QUS) measurements were performed in 7,447 children. The present analysis refers to the 2,267 children with genotypes and QUS data available at T0. QUS measurements were performed also two years later, during the follow-up, in 1,792 genotyped children.

Calcaneal Bone Stiffness

Calcaneal QUS measurements were performed using Lunar Achilles Insight (GE Healthcare, Milwaukee, WI, USA) [16]. In previous studies conducted on children, coefficient of variation was 1.9–3.5% [17–19]. Good values of short- and long-term interunit precision were reported in a prospective multicenter study [20,21]. Calibration of the QUS devices has been performed daily during the entire study period. Measurements were made according to the standard procedure provided by the manufacturer. The real time image of the calcaneus and the ROI parameter ensures that the measurement is accurate and alerts the examiner to perform the measure again when a child moved too much. An adapter was used for children's feet in order to get the proper position of the calcaneus.

The device estimates calcaneal bone stiffness index (SI), calculated from broadband ultrasound attenuation (BUA) and speed of sound (SOS): $SI = (0.67 \times BUA) + (0.28 \times SOS) - 420$. Precision ranged from 1.0 to 3.8% (CV) for BUA and from 0.19 to 0.30% (CV) for SOS [22]. The intermediate values BUA and SOS to calculate SI were retained and registered in the database only in few centers and are available only for 878 children (T1). Both feet were measured once (100% of measures) and the mean SI of both feet was calculated and used in the statistical analyses, as well as for BUA and SOS when available.

Anthropometric Measures

The measurement of weight was carried out using an electronic scale (Tanita BC 420 SMA, Tanita Europe GmbH, Sindelfingen, Germany) to the nearest 0.1 kg with children wearing indoor clothes, without shoes. Height was measured using a telescopic height-measuring instrument (Seca 225 stadiometer, Birmingham, UK) to the nearest 0.1 cm. The body mass index (BMI) was calculated as weight (in kg) divided by height squared (in m).

Genotyping

Tagging SNPs of *NMU* gene were selected from the release 2.0 Phase II data of the HapMap Project (http://hapmap.ncbi.nlm.nih.gov/) using the Tagger program of Haploview software (v4.1) [23]. Selection criteria included $r^2 \leq 0.8$ and a minor allele frequency (MAF) ≥ 0.05 in Caucasians. *NMU* gene spans 56,156,162-56,197,222 bp (NCBI.36) on chromosome region 4q12. We selected a region that includes the third block (56,187,256-56,193,006) containing eight SNPs. Of these, three tag SNPs (rs6827359, rs12500837, rs9999653) located in intronic regions were genotyped. Two missense coding SNPs in *ADRB2* gene, rs1042713 (Arg16Gly) and rs1042714 (Gln27Glu), were selected according to previous literature on obesity risk [24,25].

Saliva samples collected (Oragene DNA Self-Collection Kit, OG-300/OG-250; DNA Genotek Inc., Kanata, Ontario, Canada) from participating children were shipped to the central laboratory at the University of Glasgow for DNA extraction [14].

Variants of *NMU* gene were genotyped at Fondazione "Giovanni Paolo II" by a multiplexed end-point assay that detects variants of a single nucleic acid sequence. The allelic discrimination was performed by 7500 Fast Real-Time System (Applied Biosystems) using 96-well reaction plate with standard reagents and standard protocols. Result analysis was made by SDS v1.4 software (Applied Biosystems). Variants of *ADRB2* gene were genotyped at the University of Glasgow using Taqman assays (Applied Biosystems, Warrington, UK). Genotype calls were made by the analysis software (StepOne v2.1; Applied Biosystems). The genotyping success rate of the five variants examined was on average 97.6%. A random 5% repeated selection of samples for each SNP was genotyped again with 100% concordance.

Blood Measures

Children participating in the IDEFICS survey were asked to participate, on voluntary basis, in blood drawing. Serum/plasma samples were stored at −80°C [26]. Serum calcium was determined by a standard photometric test (o-Cresolphtalein) with a Roche Cobas Integra 800 (Roche, Mannheim, Germany). Serum cross-linked collagen N-telopeptides (β-CrossLaps) and vitamin D (25(OH)D) were measured with an electrochemiluminescence immunoassay using a Roche Modular E170 analyzer (Roche). Serum leptin was determined by radioimmunoassay (Mediagnost GmbH, Reutlingen, Germany) [27].

Statistical Analysis

The distribution of each polymorphism was assessed for deviation from Hardy-Weinberg equilibrium with chi-square test. General linear model analysis was applied to test the associations between SI and gene variants with SAS software (v9.2 for Windows, Cary, NC: SAS Institute Inc. 2002–2008) adjusting for age, sex, country and, only for T1 measurements, intervention (control *vs* intervention region). For each SNP, codominant, dominant and recessive models were tested. To select the best genetic model, we choose the genotype showing the most significant results after correction for multiple comparisons using false discovery rate (FDR) assessment. The resulting nine p values were imported in SAS and tested using PROC MULTTEST (INPVALUES option), which converts them in FDR adjusted values, to allow the use of standard significance cut off ($p<0.05$). The Haplo.stats package (v1.4.4; http://cran.r-project.org/web/packages/haplo.stats/index.html) was used to estimate the *NMU* haplotype frequencies (haplo.em function) and to verify the associations between haplotype and phenotype (haplo.glm function). The most prevalent haplotype was chosen as reference. Only the haplotypes with frequencies greater than 1% were taken into consideration for association analyses. Age, sex, country (T0) and intervention (T1 analysis) were considered as covariates. Codominant model was tested as main model, then dominant and recessive models were also tested. To investigate if an association between genotypes and SI is driven by bone mineral density rather than by trabecular structure, supplementary analyses on BUA and SOS parameters were performed in the subgroup of children with available data on these parameters.

To verify the gene-gene interaction, an interaction analysis was performed, testing the model with the two SNPs and their interaction term in the general linear model analysis. Differences in SI values were then verified between carriers and non-carriers of the most associated genotypes of both *NMU* and *ADRB2* genes.

To investigate the effect of age, sex and bone related biomarkers, further analyses were performed. The association between genotypes and SI were verified at different ages (preschool and primary school children, less than or higher than/equal to 6 years) [11] in both genders. Sex-specific SI trend during the growth was analyzed for each genotype. A locally weighted regression (LOESS) was used to test the assumption of a linear *vs.* nonlinear relationship between SI and age. SI of each child at T0 were plotted in a graph (PROC SGPLOT with LOESS statement in SAS) using a scatterplot smoothing method which automatically determines the optimal smoothing parameter [28,29]. Adjustment for BMI or weight was performed using SI residuals obtained after regression with BMI or weight as covariates.

To give more insights in biological mechanisms, gene-environmental interactions were tested using some bone related variables as calcium (n = 605), 25-hydroxy vitamin D (n = 590), leptin (n = 252) and beta-crosslaps (n = 592) levels in serum.

Results

Population Characteristics

Characteristics of the study population (N = 2,267, boys 52.4%) are listed in table 1. All genotype groups were in Hardy-Weinberg equilibrium and minor allele frequencies (MAF) resulted similar to values reported in HapMap database for Caucasians (Table 2). Five haplotypes were inferred with frequencies higher than 1% (range 2.2%–43.3%; Table 3).

Associations between NMU and SI

Using FDR correction, four models resulted significantly associated with SI among nine tested ($p = 0.05$; Table 4) at T0. SI values were 1.5–2.5 points lower in homozygotes for the variant allele C of both rs6827359 (raw $p = 0.006$) and rs12500837 (raw $p = 0.023$), as well as in homozygotes for major allele C of rs9999653 (both dominant and codominant model, raw $p = 0.014$ and 0.020, respectively), compared to carriers of the opposite allele. The use of further covariates such as BMI or weight, plausibly related to both genotypes and SI, did not change the results. The phenotype variance explained by rs6827359 CC was 0.30%, while age plus sex explained 0.12% of variance.

The most prevalent haplotype was TTC (Table 3), then it was used as referent haplotype. Subjects carrying the CCT haplotype (containing the unfavorable allele C of both rs6827359 and rs12500837) had lower SI than those carrying the most prevalent one. This result obtained using standard additive model analysis was statistically significant ($p = 0.04$, decreased value of 1 point for each haplotype copy). Dominant and recessive models were also tested, without significant results. Homozygotes for the haplotype containing only one unfavorable allele in rs6827359 (CTT/CTT) also showed lower SI values (−2.2, $p = 0.055$, homozygosis prevalence 6.2%; Table 4).

Data on QUS measurements at T1 did not reach the statistical power needed to confirm the data, although SI values were concordantly decreased in all three unfavorable genotypes (data not shown).

NMU-ADRB2 Interaction

Significant association between *ADRB2* genotypes and SI were not found. However, gene-gene interaction analysis considering both genotypes and their mathematical product revealed a statistically significant effect also for their interaction term ($p = 0.025$; table 4). Carriers of both the unfavorable genotypes (rs6827359 CC of *NMU* and rs1042713 GG of *ADRB2*, n = 186) were then compared with non-carriers, showing a larger difference in SI than single rs6827359 (−3.9, n = 1928, $p<0.0001$). The presence of both variants explained 0.64% of the phenotype variance. No significant effect was observed for SI values at T1.

Table 1. Population characteristics.

Variables	T0			T1		
	(boys = 52.4%)			(boys = 52.3%)		
	N	Mean	SD	N	Mean	SD
Age [years]	2267	6.2	1.8	1792	8.3	1.8
Body Mass Index [kg/m^2]	2267	16.3	2.2	1792	16.5	2.5
Weight [Kg]	2267	23.3	6.8	1792	24.0	7.2
Height [cm]	2267	118.5	13.0	1792	119.2	12.8
Stiffness index (mean of both feet)	2267	79.6	13.5	1792	82.9	13.5
Broadband ultrasound attenuation (BUA) (mean of both feet) [dB/MHz] (T1)	NA	NA	NA	865	88.2	16.7
Speed of sound (SOS) (mean of both feet) [m/sec] (T1)	NA	NA	NA	865	1591.5	41.5
Calcium (serum) [mmol/l]	605	2.51	0.10	NA	NA	NA
25-hydroxy vitamin D (serum) [ng/ml]	590	18.26	6.80	NA	NA	NA
Leptin (serum) [ng/ml]	252	5.10	5.35	NA	NA	NA
Beta-crosslaps (serum) [ng/ml]	592	1.18	0.27	NA	NA	NA

NA = Not Available.

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Sex-specific SI Trends during Growth

The interaction effect of categorical age (<6 or ≥6 years) and sex on SI was nominally significant ($p = 0.05$). Therefore, the SI differences between double opposite homozygotes in rs6827359 and rs1042713 polymorphisms were also tested in subgroups of children stratified for age and/or sex. The differences remained significant in both age classes and in both sexes. However, a further stratified analysis combining sex *per* age subgroups showed a large effect for younger girls (−7.4 points for double risk homozygotes, $p = 0.005$, p for interaction = 0.11), which was statistically significant yet after FDR adjustment ($p = 0.021$, using p values from the 4 subgroups of age class *per* sex), despite the decreased sample size (Table 5). The use of BMI or weight as covariates did not change the results.

SI trends during age among carriers and non-carriers of double homozygosis conditions in the subgroups of girls and boys is depicted in Figure 1. Despite the similar sample size, trends in subgroups of boys (panel A) appear to be parallel, while girls (panel B) carrying both risk genotypes showed a curvilinear trend, mainly at preschool age. The trend did not become linear after adjustment for BMI or weight.

Bone Related Parameters

BUA and SOS were available in a limited subgroup of subjects collected at T1 (n = 865). As SI, SOS values were decreased in carriers of all three unfavorable NMU genotypes, although non-significantly. Moreover, a synergistic effect of NMU and ADRB2 genotypes was observed (p for interaction = 0.01), with carriers of double unfavorable variants having a large decrease in SOS values (−10.7, $p = 0.021$) in comparison with non-carriers. The difference resulted larger in children younger than 6 years (−26.3, $p = 0.008$; p for interaction = 0.017, n = 202). No significant effect was observed for BUA values.

In a subsample ranging from 252 to 605 subjects where biomarkers were measured, homozygosity for the C allele of *NMU* rs9999653 was inversely associated with serum calcium levels, while GG genotypes of both *ADRB2* polymorphisms were associated with lower levels of β-Crosslaps ($p = 0.01$ for all). However, none of these parameters was associated with SI in this subsample (data not shown).

Discussion

This is the first population study investigating the association between *NMU* gene and bone health and its interaction with a gene in the linked sympathetic nervous pathway involved in bone

Table 2. Allele frequencies and Hardy-Weinberg Equilibrium of *NMU* and *ADRB2* gene polymorphisms (N = 2,267).

	SNP	Major:minor	Homozygous major allele	Heterozygous	Homozygous minor allele	*p* HWE	MAF	CEU
NMU	rs6827359	T:C	26.9%	48.8%	24.3%	0.11	0.49	0.40
NMU	rs12500837	T:C	57.3%	36.7%	6.0%	0.91	0.24	0.21
NMU	rs9999653	C:T	21.5%	49.1%	29.4%	0.42	0.54	0.49
ADRB2	rs1042713	G:A	37.0%	47.6%	15.4%	0.90	0.39	0.32
ADRB2	rs1042714	C:G	37.5%	47.2%	15.3%	0.62	0.39	0.46

CEU:CEPH (Utah Residents with Northern and Western European Ancestry) from International Hapmap Project.

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Table 3. Haplotypes and haplotype frequency of the 3rd *NMU* block (N = 2,267).

Haplotype			Haplotype frequency[a]
rs6827359	**rs12500837**	**rs9999653**	**Total**
T	T	C	43.3%
C	T	T	24.4%
C	C	T	21.9%
T	T	T	8.2%
C	C	C	2.2%

[a]Rare haplotypes with frequency lower than 1% were not considered (CTC, TCC and TCT, accounting for 0.05%).
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Table 4. Differences in bone stiffness index (Δ SI) between risk and non-risk genotype carriers at T0.

Gene	SNP	Risk genotype	Frequency	Δ SI	*P*[a]
NMU	rs6827359 T/C	CC	24.3%	−1.8	0.006[b]
NMU	rs12500837 T/C	CC	6.0%	−2.6	0.023[b]
NMU	rs9999653 C/T	CC[c]	21.5%	−1.5	0.014[b]
NMU	H3[d]	CCT/x	39.7%	−1.0	0.04
NMU	H2[d]	CTT/CTT	6.2%	−2.2	0.055
ADRB2	rs1042713 G/A	GG[c]	37.0%	−0.9	0.09
ADRB2	rs1042714 C/G	GG	15.3%	−0.6	0.37
*NMU*ADRB2*	rs6827359*rs1042713	CC+GG	8.4%	−3.9	<0.0001

[a]Adjusted for age, sex and country.
[b]Model selected according to the highest association after FDR correction (PROC MULTTEST in SAS software).
[c]Homozygotes for wild-type (instead of variant) allele were shown to concordantly retain the genotypes with lower values.
[d]Carriers of Haplotype TTC as reference.
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health regulation. Genetic variants of *NMU* gene are associated with SI in European children of IDEFICS population, with allele C of SNP rs6827359 showing the most significant decrease in SI. Haplotype analysis confirmed the involvement of *NMU* gene and, in particular, of SNP rs6827359, in bone health. Furthermore, there was a significant interaction between *NMU* and *ADRB2* gene, with double risk homozygous children showing a decrease of 3.9 point in SI with respect to double opposite homozygotes. The effect appeared to be more evident in younger girls, in whom a curvilinear SI trend during growth was observed. Subgroup analyses showed that the effect on SI was mainly driven by the effect on its component SOS and that the genotype-SI association was not mediated by bone related biomarkers.

NMU is a hypothalamic neuropeptide, involved in the regulation of various metabolic functions [4]. Recently, NMU was suggested to be involved also in bone health determination [5]. One of the main systems regulating bone formation involves leptin that inhibits bone formation by binding to its receptors located in hypothalamus and thereby activating the SNS. This process requires the expression of ADRB2 in osteoblasts, which mediates several effects of catecholamine hormones and neurotransmitters in bone [30]. The inhibition of bone formation through a hypothalamic relay suggested that molecules affecting energy metabolism in the hypothalamus, such as NMU, could also modulate bone mass. Using transgenic mice, Sato et al. showed that NMU acts as a modulator of leptin-SNS-ADRB2 regulation of bone formation, through a central relay and via an unidentified pathway. *NMU*-deficient mice had high bone mass due to an increase in bone formation that was reversed by a natural agonist for the NMU receptor. Furthermore, transgenic mice null for one allele of both *NMU* and *ADRB2* genes showed a more pronounced increase in bone mass, suggesting an interaction between NMU and ADRB2 [5–7]. We here demonstrated this phenomenon in children, showing that different SI values are associated with different *NMU* genotypes and with double homozygotes of both *NMU* and *ADRB2* risk genotypes. Thus, observations from *in vitro* data and transgenic mouse models were confirmed in humans, that NMU is involved in bone health and interacts with ADRB2 signal.

QUS is a radiation free, portable, cost-effective tool, measuring properties of bone that contribute to mechanical bone strength by sending sound waves; the more complex the bone structure, the more sound waves will be absorbed [31]. Measuring SI with QUS has value in assessing associations in children. In fact, over the last decades, many studies used QUS to study bone strength in healthy children at the calcaneus [17–19,32,33] even in preschool children [12,34] and it was suggested to predict the risk of osteopenia or fragility fracture [33,35]. Calcaneus is the most popular site for QUS measurement since it consists for 90% of trabecular bone and has a high turnover rate and therefore will show bone metabolic changes first [1]. Moreover, using transaxial transmission, the QUS parameters are related to bone density and structure and not to cortical thickness, which is an advantage when examining children and adolescents, since cortical thickness varies a lot during growth [36]. Finally, the safe and portable nature of the method will allow designing feasible screening programs from school to school, then calcaneus SI could became a useful marker for public health interventions.

The SI is derived from the measured values BUA, which largely reflects bone mineral density, and SOS, which reflects elasticity and density due to trabecular connectivity [37]. Unfortunately, BUA and SOS were available only at T1 and only for a subgroup of children. Although the limited sample size, SOS showed a strong association with double homozygosity of risk alleles. This result suggests that NMU and ADRB2 are involved more in microarchitecture formation than in mineral content accrual, confirming the data from Sato et al. [5], showing a prevalent role for NMU in osteoblast proliferation rather than in their function. In fact, in *NMU*-null mice they observed higher osteoblast numbers in the presence of a normal mineral apposition rate.

Despite the decreased sample size of subgroup analyses of age and sex, in girls less than 6 years significant associations were found between genotypes and SI, concordantly with the overall sample. Analyzing trends across ages, in girls a curvilinear trend was observed for carriers of both risk genotypes, while in boys SI trends appeared to be linear and parallel. Since sample sizes of boys and girls are similar, the reason of non-linearity should be mainly attributed to the major dispersion of SI values observed in pre-school girls, which suggest the involvement of other factors at this age. Anyway, the stronger association observed in younger girls could be determined by the NMU-SNS pathway and this suggests to deeply investigating the sex-specific factors potentially involved at earlier ages. These observations could be of relevance, since bone peak at early ages has been associated with future osteoporosis [2,38]. A link between the hypothalamic neuropeptide NMU and gonadotropin secretion is known [39], which however takes places at later ages. Gender differences were also

Table 5. Bone stiffness index values in children with different combination of *NMU* rs6827359 and *ADRB2* rs1042713 alleles.

rs6827359* rs1042713	Overall			Boys						Girls					
				<6 years			≥6 years			<6 years			≥6 years		
	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD
00 (TT+AA)	1006	80.4	0.4	241	81.1	0.9	278	80.3	0.7	230	81.4	1.0	257	79.0	0.7
01 (TT+GG)	601	80.0	0.5	141	80.0	1.2	189	80.1	0.8	118	81.1	1.2	153	79.3	0.9
10 (CC+AA)	321	79.7	0.7	62	82.7	1.8	113	79.9	1.0	60	79.3	1.8	86	77.8	1.2
11 (CC+GG)	186	76.3	0.9	40	76.4	2.3	61	77.3	1.4	29	73.5	2.6	56	76.8	1.5
***P* for interaction**	0.025			0.120			0.262			0.105			0.583		
00+01+10	1928	80.1	0.3	444	81.1	0.7	579	80.2	0.5	408	80.9	0.7	496	78.9	0.5
11 (CC+GG)	186	76.3	0.9	40	76.4	2.3	61	77. 3	1.4	29	73.5	2.6	56	76.8	1.5
11 vs 10+01+00 Delta	−3.9			−4.7			−2.9			−7.4			−2.1		
***P* value**	0.0001			0.045			0.051			0.005			0.189		

Stiffness index values are least square means computed in a glm analysis using the variable with the four genotypes as independent variable. P for interaction was computed for CC*GG. *P* value for the association with double homozygotes for risk alleles were reported (heterozygotes were excluded).
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reported in NMU-deficient mice [5], where the increase in bone formation was however more prominent in male than in female mice.

Finally, we investigated possible mechanisms underlying the association between *NMU/ADRB2* gene and bone stiffness, by measuring biological markers related to bone metabolism in serum samples of a children subgroup. Some associations were found with calcium or β-CrossLaps levels, bone turnover markers, however they were present for SNPs less associated with SI. Furthermore, an association between SI and these biomarkers was not found, due to limited statistical power in the subgroup of genotyped subjects with biomarkers measured.

The study has some limitations.

Indeed, being the first study in humans suggesting an effect on *NMU* on bone health, it needs confirmation. The GWAS performed on bone health measured with different techniques did not reveal any effect for SNPs near *NMU* region. However, GWAS are focused on a relatively limited selection of polymorphisms which cannot be in linkage with all other known polymorphism and report only highly significant associations, as well as do not study neither all genetic model nor the interactive effects among genotypes [9]. Furthermore, heritability of QUS traits ranged from 0.42 to 0.57 [40], allowing further researches on polymorphisms with minor effects or on plausible gene-gene interactions.

The main strength of our study is a strong biological plausibility, since our results reproduce those observed in mice [5]. Moreover, it is performed on a large sample recruited in eight countries and it is focused on children, in which environmental exposure time is short and genetics should have more impact than expected.

In conclusion, our study in European children suggests an involvement of NMU-SNS pathway in bone stiffness, mainly in bone microarchitecture, more evident in preschool girls. The identification of genetic markers in the NMU pathway could be helpful in planning therapies for bone-loss disorders or metabolic diseases using novel NMU receptors inhibitors or NMU analogs [41], as well as in finding novel specific targets for preventive or therapeutic interventions.

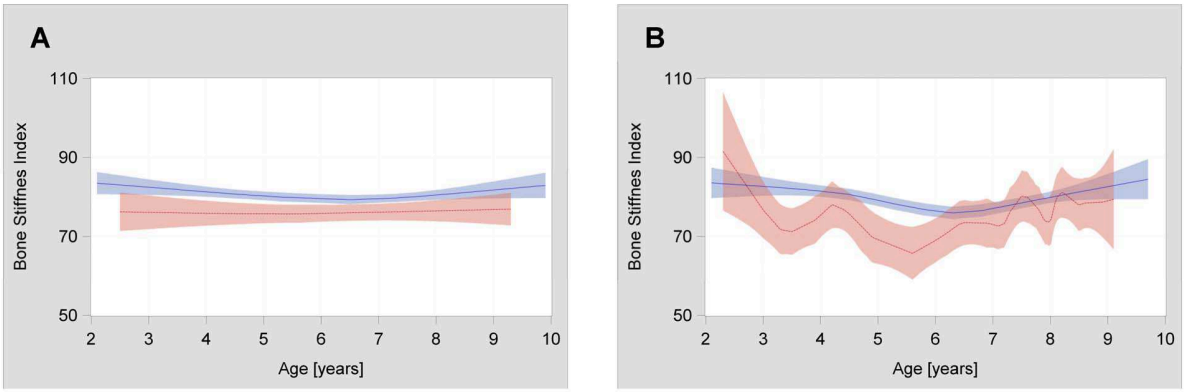

Figure 1. Distribution of SI values at different ages, stratified for genotypes and sex. SI values and their 95% confidence intervals at different ages for homozygotes of both *NMU* rs6827359 and *ADRB2* rs1042713 risk alleles (CC+GG, dark grey) and homozygotes for non-risk alleles (TT+AA, light gray) in subgroups of boys (panel A, n = 620, carriers of CC+GG = 101) and girls (panel B, n = 572, carriers of CC+GG = 85). Graph was obtained using SAS software (PROC SGPLOT with LOESS statement, see text). Local regression method implies that statistical power decreases at extreme *x* values (larger confidence intervals).
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Author Contributions

Conceived and designed the experiments: WA LI. Performed the experiments: DC MESB ACK. Analyzed the data: FG KB. Wrote the paper: FG LI. Acquisition of data: DH YK SM DM LAM YPP PR AS SS IS TV. Revising and approving final version of manuscript: FG DC WA MESB KB DH ACK YK SM DM LAM YPP PR AS SS IS TV LI.

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Appendix F

Relationship Between Markers of Body Fat and Calcaneal Bone Stiffness Differs Between Preschool and Primary School Children: Results from the IDEFICS Baseline Survey

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ORIGINAL RESEARCH

Relationship Between Markers of Body Fat and Calcaneal Bone Stiffness Differs Between Preschool and Primary School Children: Results from the IDEFICS Baseline Survey

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Abstract The aim of this study was to investigate the relationship between markers of body fat and bone status assessed as calcaneal bone stiffness in a large sample of European healthy pre- and primary school children. Participants were 7,447 children from the IDEFICS study (spread over eight different European countries), age 6.1 ± 1.8 years (range 2.1–9.9), 50.5 % boys. Anthropometric measurements (weight, height, bioelectrical impedance, waist and hip circumference, and tricipital and subscapular skinfold thickness) as well as quantitative ultrasonographic measurements to determine calcaneal stiffness index (SI) were performed. Partial correlation analysis, linear regression analysis, and ANCOVA were stratified by sex and age group: preschool boys ($n = 1{,}699$) and girls ($n = 1{,}599$) and primary school boys ($n = 2{,}062$) and girls ($n = 2{,}087$). In the overall study population, the

This study was conducted on behalf of the IDEFICS Consortium. The authors have stated that they have no conflict of interest.

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average calcaneal SI was equal to 80.2 ± 14.0, ranging 42.4–153. The results showed that preschool children with higher body fat had lower calcaneal SI (significant correlation coefficients between −0.05 and −0.20), while primary school children with higher body fat had higher calcaneal SI (significant correlation coefficients between 0.05 and 0.13). After adjusting for fat-free mass, both preschool and primary school children showed an inverse relationship between body fat and calcaneal stiffness. To conclude, body fat is negatively associated with calcaneal bone stiffness in children after adjustment for fat-free mass. Fat-free mass may confound the association in primary school children but not in preschool children. Muscle mass may therefore be an important determinant of bone stiffness.

Keywords Bioimpedance measurement · BMI · Body fat · Bone stiffness · Calcaneal quantitative ultrasonography · Fat-free mass

Bone development is one of the key processes characterizing growth during childhood and adolescence [1]. Understanding this developmental process is of crucial importance not only for the treatment of pediatric bone disorders but also for early prevention strategies to improve bone strength and to prevent osteoporosis later in life. Prevention strategies at this young age are crucial as the peak bone mass is built up during growth in childhood, puberty, adolescence, and early adulthood [2–4].

Bone development is influenced, among other things, by the development of other compartments of the body, i.e., lean mass and fat mass. However, whether overweight and obese people have better or worse bone health is yet unclear. Several studies have shown that overweight and obese children have higher levels of bone mineral content (BMC) and bone mineral density (BMD) [5–7], while others have observed the opposite [8–10]. Nevertheless, the number of studies investigating the relation between body fat and bone strength in very young children are limited, and the studies including preschool children do not differentiate results across different age groups [5, 9]. To provide more in-depth knowledge on the relation between markers of body fat and bone strength in young children, this study investigated a large sample of European, healthy, preschool and primary school children.

To study bone strength in these children, a radiation-free, portable, cost-effective tool was used, i.e., quantitative ultrasound sonography (QUS). The technique measures properties of bone that contribute to mechanical bone strength by sending sound waves; the more complex the bone structure, the more sound waves will be absorbed [11–13]. In children, QUS at the calcaneus predicts the risk of osteopenia or fragility fracture [14, 15]. Moreover, using transaxial transmission, the QUS parameters are related to bone density and structure and not to cortical thickness, which is an advantage when working with children and adolescents since cortical thickness varies during growth [8, 16, 17]. Over the last decades, many studies have used QUS to study bone strength in healthy children. However, assessments at different peripheral skeletal sites were performed, e.g., at the calcaneus [3, 10, 13, 14, 18–29], the proximal phalanges of the hand [30, 31], and the tibial midshaft alone [32] or along with the distal third of the radius [8, 12, 33–35]. The calcaneus is the most popular site for QUS measurement since it consists of 90 % trabecular bone, has a high turnover rate, and therefore, will show bone metabolic changes first [1]. So far, the number of studies using QUS to investigate bone-related parameters in preschool children is limited [28, 31, 34, 35].

Therefore, we aimed (1) to describe calcaneal bone stiffness data for a large cohort of healthy European preschool and primary school children and (2) to assess in these children the relation between markers of body fat and bone status assessed as calcaneal bone stiffness.

Methods

Subjects

The IDEFICS (Identification and Prevention of Dietary- and Lifestyle-Induced Health Effects in Children and Infants; www.ideficsstudy.eu) multicenter study is an integrated project within the Sixth Framework Programme of the European Commission. It is a cohort study of pre- and primary school children in eight European countries (Belgium, Cyprus, Estonia, Germany, Hungary, Italy, Spain, Sweden). It longitudinally investigates the etiology of diet- and lifestyle-related diseases and disorders in a large sample of children and develops and evaluates a primary prevention program focusing on obesity. Research goals and instruments have been described in detail [36]. During the IDEFICS baseline survey (September 2007–May 2008), anthropometric and calcaneal QUS measurements were performed in 7,447 children from the eight participating countries (age 6.1 ± 1.8 years, range 2.1–9.9 years, 50.5 % boys). The study population was divided in two age groups: preschool children ($2 < \text{age} < 6$ years) and primary school children ($6 \leq \text{age} < 10$ years). A questionnaire was completed by the parents, collecting, among other things, information about their maximal educational attainment, which was classified according to the International Standard Classification of Education for both parents of each child. The study was conducted according to the guidelines of the Declaration of

Helsinki, and approval of the ethical committee was obtained for each survey center. Informed written consent for the study was obtained from the parents of all participating children who gave oral consent.

Anthropometric Measurements and Definition of Weight Status

A detailed description of the anthropometric measurements adopted in the IDEFICS study, including intra- and interobserver reliability, has been recently published [37]. In all study centers standardized procedures were followed for the measurements and all study nurses followed a central training. Height and weight were measured, respectively, with a standard clinical Seca 225 stadiometer (Seca, Hamburg, Germany) to the nearest 0.1 cm and a scale (BC 420 SMA; Tanita, Amsterdam, The Netherlands) to the nearest 0.1 kg. The children wore only underwear and T-shirts. The Tanita scale (adapted to the small foot size of children) also measured leg-to-leg impedance (Ω). The scale was calibrated and did not need further calibration; also, the Seca stadiometer needed neither maintenance nor further calibration. Body mass index (BMI) was calculated according to the following formula: BMI = weight (kg)/height^2 (m^2). For each child, z scores of weight, height, and BMI were determined using the LMS method of Cole (with British reference population) [38]. Underweight, normal weight, overweight, and obesity were defined using the International Obesity Taskforce (IOTF) BMI categories [39]. Fat-free mass (FFM) was calculated using the Tyrrell et al. [40] formula: FFM (kg) = 0.31 × height^2 (cm^2)/impedance (Ω) + 0.17 × height (cm) + 0.11 × weight (kg) + 0.942S − 14.96 (S = 1 for girls, S = 2 for boys).

Waist and hip circumferences were measured with the children standing using Seca 200 inelastic tape (precision 0.1 cm, range 0–150 cm). This tape did not need any calibration. Waist-to-hip ratio was calculated as an indicator of central obesity, and waist-to-height ratio was used as a normalized index of body fat distribution. Skinfold thickness was measured at two sites using Holtain Tanner/Whitehouse skinfold calipers (Holtain, Crosswell, UK; range 0–40 mm). Tricipital, halfway between the acromion and the olecranon process at the back of the arm, and subscapular skinfold thickness, about 2 cm below the tip of the scapula, at an angle of 45° to the lateral side of the body, were measured twice (according to the international standards for anthropometric assessment [41]). The mean of the two measurements was calculated. The sum of the mean tricipital and mean subscapular skinfold was used as a marker of body fat. The calipers were calibrated every morning and additionally when dropped by means of a calibration block of 20 mm. The intra- and interobserver reliability values in anthropometric measurements in children were assessed in different participating IDEFICS centers. The intraobserver technical error of measurement (TEM) was between 0.12 and 0.47 mm for skinfold thickness and between 0.09 and 1.24 cm for circumference measurements. Intraobserver reliability was 97.7 % for skinfold thickness (triceps, subscapular, biceps, suprailiac) and 94.7 % for circumferences (neck, arm, waist, hip). Interobserver TEMs were for skinfold thicknesses between 0.13 and 0.97 mm and for circumferences between 0.18 and 1.01 cm. Interobserver agreement as assessed by the coefficient of reliability for repeated measurements of skinfold thickness and circumferences was above 88 % in all countries [37].

Calcaneal Bone Stiffness

For all children and in all IDEFICS centers, calcaneal QUS measurement was performed with Lunar Achilles Insight® (GE Healthcare, Milwaukee, WI), which had previously been used in other studies with children [18, 27, 29, 42]. However, it has to be mentioned that the children in those earlier studies were at least 5 years old or older. The coefficient of variation for in vivo calcaneus measurement by QUS (Lunar Achilles device) is 1.9–3.5 % in children [18, 29, 42]; precision as given by the manufacturer is 1.7 % [43]. Economos et al. [43] showed that these devices (Lunar Achilles) are internally consistent and that different machines may be used over time to provide reliable measurements of changes in bone quality. Hans et al. [44] assessed the interunit variations in vitro and in vivo of an ultrasound instrument, the Lunar Achilles system, used in a French prospective multicenter study (similar type of device and similar study design as in this IDEFICS study). They found that the short-term and long-term interunit precision values were good, both in vitro and in vivo, and concluded that their results provided increased confidence in multicenter trials where ultrasonic data are pooled.

The device estimates calcaneal bone stiffness index (SI), calculated from the parameters broadband attenuation (BUA) and speed of sound (SOS): SI = (0.67 × BUA) + (0.28 × SOS) − 420. In the IDEFICS baseline survey, only the SI values were stored by the device; therefore, the BUA and SOS parameters were not available. The real-time image of the calcaneus and the region of interest (ROI) ensures that the measurement is accurate. Daily calibration was done during the entire study period, and measurements were made according to the standard procedure provided by the manufacturer. An adapter was used for the children's feet in order to get the proper position of the calcaneus. When a child moved too much during the measurement, no result was given by the QUS device—an error sign indicated that the measurement failed—and the measurement had to be redone. The overall QUS

measurement took about 10 min per child. Both feet were measured once, and the mean SI of the two measurements was calculated and used in the statistical analyses.

Statistics

Since sex and age are important determinants of body fat, all statistical analyses were stratified by sex and by age group: preschool boys ($n = 1{,}699$), preschool girls ($n = 1{,}599$), primary school boys ($n = 2{,}062$), and primary school girls ($n = 2{,}087$). Independent samples t tests were used to compare differences in SI between the predefined groups. Partial correlation analyses were carried out to determine the correlation between SI and other continuous variables after controlling for the cluster design (controlling variable was "survey center"). The relationship between body fat (using different anthropometric parameters as markers of body fat) and calcaneal SI was analyzed using multiple linear regression models. In the first set of models the relationship between markers of body fat and calcaneal stiffness was corrected for height and age; in the second set of models the relationship was corrected for height, age, and FFM. When using BMI as a marker for body fat, height was not included as a covariate. Moreover, body weight and not body weight z score was used in the regression analyses since the analyses were corrected for height and age. One-way analysis of covariance (ANCOVA) was used to analyze the impact of body fat on calcaneal SI, using the IOTF BMI category as a fixed factor (four categories: underweight, normal weight, overweight, obese). The first set of ANCOVAs was corrected for height and age. In the second set, also a correction for FFM was performed. All analyses were performed with the PASW Statistics program, version 18.0.0 (SPSS, Inc., Chicago, IL), and $p < 0.05$ was considered statistically significant.

Results

Table 1 shows the mean and standard deviation of the body mass and body composition parameters by sex and age group. Girls had significantly lower weight, height, waist circumferences, and FFM but thicker skinfolds compared to boys. Moreover, primary school girls had significantly lower BMI z scores than boys. Table 1 also shows data for calcaneal SI by sex and age group. In preschool children, calcaneal SI was higher in girls compared to boys; however, this difference was not statistically significant (p 0.083). In primary school children, the opposite was seen: SI was lower in girls compared to boys (p 0.022). When comparing the age groups, SI was higher in preschool children compared to primary school children both in boys (p 0.010) and in girls ($p < 0.001$). No significant association was found between educational attainment of both parents and the SI of the children (data not shown). Therefore, this variable was not taken into account in the regression analyses.

Table 2 shows the correlations between SI and age and between SI and different body mass and body composition parameters after controlling for the cluster design. Concerning age, an inverse association with SI was found in preschool children ($r = -0.075$ for boys and $r = -0.087$ for girls), while the association was positive in primary school children ($r = 0.106$ for boys and $r = 0.127$ for girls). For height, a negative association with SI was found in the preschool children, whereas in primary school girls a small, positive association was found. The same pattern was also observed for other anthropometric parameters: a significant negative association in preschool children and a significant positive association in primary school children (Table 2). For skinfold thickness, also significant negative associations were found in preschool children, whereas no significant associations were found in primary school children, except for the subscapular and the sum of skinfold thicknesses in primary school girls. Also for FFM, a negative association was found with SI in preschool children, while a positive association was found in primary school children. Significant positive associations were found between SI and waist to hip and waist to height ratios in all groups, except in preschool girls.

Figure 1 shows the SI data in children grouped on a yearly basis. This figure illustrates the negative correlation between age and SI in preschool children and the positive correlation between age and SI in primary school children.

Table 3 shows the association between calcaneal SI and different markers of adiposity (entered one by one in the model) after correcting for height and age (model 1) and for height, age, and FFM (model 2). Body weight, BMI, and skinfold thickness were negatively associated with SI in preschool children; and this remained or became even stronger after adjustment for FFM, with the exception of BMI for which no significant association remained. In primary school children, the significant, positive association between SI and body weight as well as BMI inverted to a significant negative association for weight and no association for BMI after adjustment for FFM. Adjustment for FFM resulted in significant negative associations between skinfold thicknesses and calcaneal SI in primary school children, whereas no association was found without correction for FFM.

Figure 2 shows for each sex and age group the difference in calcaneal SI by different IOTF BMI categories for the first model (correction for height and age) and for the second model (correction for height, age, and FFM). This figure illustrates that in preschool children body fat is negatively

Table 1 Descriptive characteristics of the studied children by sex and age groups, as well as *p* values for the sex difference within each age group

	Boys 2–6 years (n = 1,699)	Girls 2–6 years (n = 1,599)	p (sex difference)	Boys 6–9 years (n = 2,062)	Girls 6–9 years (n = 2,087)	p (sex difference)
Calcaneal stiffness index	80.9 ± 15.0	81.8 ± 16.8	0.083	79.7 ± 12.0	78.9 ± 12.4	0.022
Body weight (kg)	18.3 ± 3.6	17.8 ± 3.5	<0.001	27.6 ± 6.4	27.0 ± 6.2	0.001
Body weight z score	0.3 ± 1.1	0.3 ± 1.1	0.107	0.6 ± 1.2	0.4 ± 1.1	<0.001
Height (cm)	106.9 ± 7.9	105.9 ± 7.8	<0.001	127.9 ± 7.3	126.7 ± 7.2	<0.001
Height z score	0.5 ± 1.1	0.5 ± 1.1	0.410	0.6 ± 1.1	0.4 ± 1.0	<0.001
BMI (kg/m^2)	15.9 ± 1.7	15.8 ± 1.7	0.070	16.7 ± 2.7	16.7 ± 2.7	0.419
BMI z score	0.0 ± 1.2	0.0 ± 1.1	0.841	0.4 ± 1.3	0.2 ± 1.2	<0.001
Leg-to-leg impedance (Ω)	644.4 ± 64.8	684.9 ± 69.6	<0.001	614.3 ± 64.0	658.2 ± 68.5	<0.001
Fat-free mass (kg)	12.7 ± 2.7	11.1 ± 2.6	<0.001	20.1 ± 3.2	18.2 ± 3.0	<0.001
Waist circumference (cm)	51.0 ± 4.3	50.4 ± 4.4	<0.001	57.2 ± 7.1	56.2 ± 7.0	<0.001
Hip circumference (cm)	57.2 ± 5.2	57.5 ± 5.2	0.117	66.9 ± 7.1	67.4 ± 7.0	0.055
Waist/hip ratio	0.89 ± 0.05	0.88 ± 0.05	<0.001	0.86 ± 0.06	0.83 ± 0.05	<0.001
Waist/height ratio	0.48 ± 0.04	0.48 ± 0.04	0.541	0.45 ± 0.05	0.44 ± 0.05	0.027
Tricipital skinfold (mm)	10.0 ± 2.6	11.3 ± 3.0	<0.001	10.8 ± 4.4	12.7 ± 4.7	<0.001
Subscapular skinfold (mm)	6.0 ± 2.1	6.7 ± 2.3	<0.001	7.0 ± 4.3	8.2 ± 4.9	<0.001
Sum of both skinfolds (mm)	15.9 ± 4.3	17.9 ± 4.9	<0.001	17.8 ± 8.4	20.8 ± 9.1	<0.001

Data are mean ± SD

Table 2 Correlation coefficients between calcaneal stiffness index and anthropometric parameters stratified by sex and age controlled for cluster design

	Boys 2–6 years	Girls 2–6 years	Boys 6–9 years	Girls 6–9 years
Age (years)	−0.075**	−0.087*	0.106*	0.127*
Height z score	−0.140*	−0.104*	0.031	0.049***
Body weight z score	−0.161*	−0.160*	0.071*	0.084*
BMI z score	−0.105*	−0.137*	0.085*	0.087*
Leg-to-leg impedance (Ω)	0.012	0.063***	−0.177*	−0.190*
Fat-free mass (kg)	−0.142*	−0.154*	0.144*	0.128*
Waist circumference (cm)	−0.085*	−0.104*	0.121*	0.130*
Hip circumference (cm)	−0.129*	−0.131*	0.093*	0.106*
Tricipital skinfold (mm)	−0.140*	−0.196*	0.021	0.039
Subscapular skinfold (mm)	−0.095*	−0.156*	0.028	0.059**
Sum of both skinfolds (mm)	−0.130*	−0.196*	0.025	0.052***
Waist/hip ratio	0.085*	0.048	0.071*	0.091*
Waist/height ratio	0.053***	0.015	0.087*	0.087*

Data are adjusted for cluster design: * $p \leq 0.001$, ** $p < 0.010$, *** $p < 0.050$

associated with calcaneal SI even after adjustment for FFM. In primary school children, adjustment for FFM inverts the relation between body fat and calcaneal SI.

Discussion

The main results of this cross-sectional study based on 7,447 European children is the strong age effect observed in the relation between markers of body fat and bone status assessed as calcaneal SI. Negative associations between markers of body fat and calcaneal SI were found in pre-school children, whereas positive relationships were found in primary school children. However, the positive association in primary school children was explained by the higher FFM or a related factor linking FFM and bone stiffness as the relation inverted after adjustment for FFM. Inconsistent results exist on the effect of obesity or body mass on bone during growth. Leonard et al. [5] assessed BMC by dual-energy X-ray absorptiometry (DXA) in 235

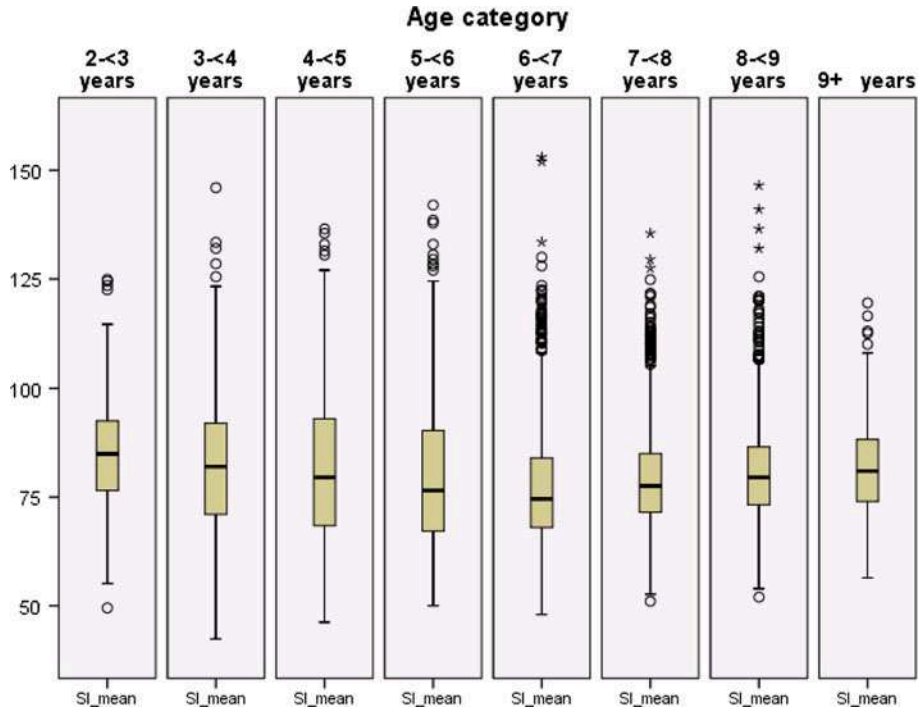

Fig. 1 Box plot of the stiffness index (SI) in children grouped on a yearly basis. *Open circles* outliers, *asterisks* extreme value

children and adolescents aged 4–20 years and concluded that obesity during childhood and adolescence was associated with increased vertebral bone density and increased whole-body bone dimensions and mass. Goulding et al. [9] examined 336 children and adolescents between 3 and 19 years old using DXA and also found that obese children had higher BMC and bone area than those with normal weight. However, they concluded that in overweight and obese children there is a mismatch between body weight and bone development during growth since their bone mass and bone area are low for their body weight. At variance with the results of the present study, Leonard et al. [5] and Goulding et al. [9] did not report a different relation between body weight and BMC in preschool children compared to primary school children. However, their sample size was much smaller compared to the IDEFICS one, which may be why they did not consider different age categories. Falk et al. [8] and Rocher et al. [10] investigated bone properties in children aged 9–12 years using QUS measurements at the tibia and radius as well as at the calcaneus, respectively. Rocher et al. [10] found higher calcaneal BUA values in obese children; however, this difference disappeared after correction for fat mass (measured by DXA), indicating that the skeletal adaptation was not sufficient to compensate for the excess load on the whole body. In contrast, Falk et al. [8] reported that obesity in boys appeared to be associated with lower SOS in the tibia and radius. Gracia-Marco et al. [6] investigated the relationship between adiposity and bone health in adolescents, using measurements by DXA and BodPod. The authors concluded that adolescents with higher levels of adiposity have greater bone mass, but this association was fully explained by higher lean mass as there was a negative association with adiposity after adjustment for FFM. In this study, the same association was found in primary school children but not in preschool children, where the association was negative before and after adjustment for FFM.

There are several possible mechanisms that may increase bone stiffness in overweight and obese children. First, hormonal factors can play a role, e.g., higher levels of circulating leptin, which acts as a growth factor, and sex hormones in obese children compared to children with normal weight. Higher concentrations of these hormones may result in larger bone size and bone mass compared with normal-weight children [5, 8, 45]. Second, differences exist in nutritional intake between obese and normal-weight children (e.g., differences in calcium intake) as well as in physical activity [8]. Next, increased mechanical loading due to increased body weight and increased lean muscle mechanical forces that are needed to move around the greater body mass may also contribute to increased bone strength and mass in obese children [5, 8, 10]. The calcaneus has a central position in supporting the weight of the body, which makes it an interesting skeletal site to study the effect of weight on bone strength [46]. Some possible hypotheses for the inverse relationship found between body weight and calcaneal SI in primary school versus preschool children found in this study are (1) that the composition of bone tissue differs in preschool children compared to older children, which results in a different

Table 3 Multiple linear regression analyses investigating the relationship between markers of body fat and calcaneal stiffness by sex and age groups

	Boys (model 1)[a]			Boys (model 2)[b]			Girls (model 1)[a]			Girls (model 2)[b]		
	B[c]	Part corr	p	B[c]	Part corr	p	B[c]	Part corr	p	B[c]	Part corr	p
Preschool children												
Body weight (kg)	−0.099	−0.057	**0.017**	−0.272	−0.096	**<0.001**	−0.211	−0.125	**<0.001**	−0.242	−0.095	**<0.001**
BMI (kg/m^2)[d]	−0.095	−0.094	**<0.001**	−0.039	−0.034	0.161	−0.145	−0.145	**<0.001**	−0.100	−0.090	**<0.001**
Waist circumference (cm)	−0.001	0.000	0.984	−0.005	−0.004	0.884	−0.054	−0.047	0.059	0.000	0.000	0.995
Hip circumference (cm)	−0.064	−0.049	**0.040**	−0.106	−0.065	**0.007**	−0.086	−0.065	**0.009**	−0.028	−0.017	0.497
Waist to hip ratio	0.064	0.062	**0.011**	0.064	0.062	**0.011**	0.019	0.018	0.473	0.018	0.017	0.501
Waist to height ratio	0.001	0.001	0.977	−0.003	−0.002	0.933	−0.053	−0.048	0.054	−0.002	−0.002	0.952
Tricipital skinfold (mm)	−0.117	−0.115	**<0.001**	−0.136	−0.124	**<0.001**	−0.180	−0.176	**<0.001**	−0.171	−0.158	**<0.001**
Subscapular skinfold (mm)	−0.072	−0.071	**0.004**	−0.095	−0.082	**0.001**	−0.140	−0.138	**<0.001**	−0.127	−0.111	**<0.001**
Sum of both skinfolds (mm)	−0.108	−0.106	**<0.001**	−0.136	−0.119	**<0.001**	−0.179	−0.175	**<0.001**	−0.174	−0.153	**<0.001**
Primary school children												
Body weight (kg)	0.111	0.077	**<0.001**	−0.186	−0.076	**<0.001**	0.098	0.070	**0.001**	−0.225	−0.092	**<0.001**
BMI (kg/m^2)[d]	0.078	0.077	**<0.001**	0.025	0.019	0.372	0.080	0.079	**<0.001**	0.021	0.016	0.445
Waist circumference (cm)	0.099	0.087	**<0.001**	−0.028	−0.018	0.414	0.095	0.085	**<0.001**	−0.032	−0.021	0.334
Hip circumference (cm)	0.058	0.046	**0.035**	−0.158	−0.088	**<0.001**	0.044	0.036	0.101	−0.200	−0.108	**<0.001**
Waist to hip ratio	0.081	0.081	**<0.001**	0.055	0.054	**0.013**	0.113	0.111	**<0.001**	0.086	0.083	**<0.001**
Waist to height ratio	0.087	0.087	**<0.001**	−0.021	−0.016	0.462	0.088	0.088	**<0.001**	−0.021	−0.016	0.462
Tricipital skinfold (mm)	−0.001	−0.001	0.953	−0.134	−0.105	**<0.001**	0.001	0.001	0.956	−0.129	−0.101	**<0.001**
Subscapular skinfold (mm)	0.009	0.009	0.695	−0.134	−0.103	**<0.001**	0.023	0.022	0.304	−0.113	−0.086	**<0.001**
Sum of both skinfolds (mm)	0.003	0.003	0.883	−0.148	−0.112	**<0.001**	0.014	0.014	0.533	−0.135	−0.100	**<0.001**

Significant p values are indicated in bold

Part corr partial correlation

[a] Model 1 included height (in cm) and age (in years) as covariates

[b] Model 2 is model 1 + fat-free mass (in kg)

[c] B is the standardized regression coefficient

[d] Models including BMI as independent variable were not corrected for height

reaction on increasing body weight; (2) that the bone needs time to adapt to the higher body weight so that there is a time lag between the skeletal adaptation and the increase in body weight; and/or (3) that the influence of hormones on bone mass is different in preschool children compared to primary school.

QUS Results in Preschool Children

Data describing QUS variables in children younger than 6 years are limited. We found no study using the same type of QUS device in preschool children as was used in the IDEFICS study (Lunar Achilles Insight). Baroncelli et al. [31] measured phalangeal QUS using a DBM Sonic bone profiler (Igea, Carpi, Italy); Nohara et al. [28] reported the use of a device called the Benus II (Japan) to determine calcaneal BMD; Christoforidis et al. [35] as well as Zadik et al. [34] used a Sunlight Omnisense device (Sunlight Medical, Tel Aviv, Israel) to measure the tibia and radius. Baroncelli et al. [31] assessed bone mineral status by phalangeal QUS in 3,044 healthy subjects 2–21 years old. In both sexes, they found that the amplitude-dependent SOS and bone transmission time increased significantly with height and weight, also in the youngest children. No negative associations were reported like those observed in preschool children in our study. Nevertheless, it is difficult to compare the two studies since the phalanges contain more cortical bone (approximately 60 %) compared to the

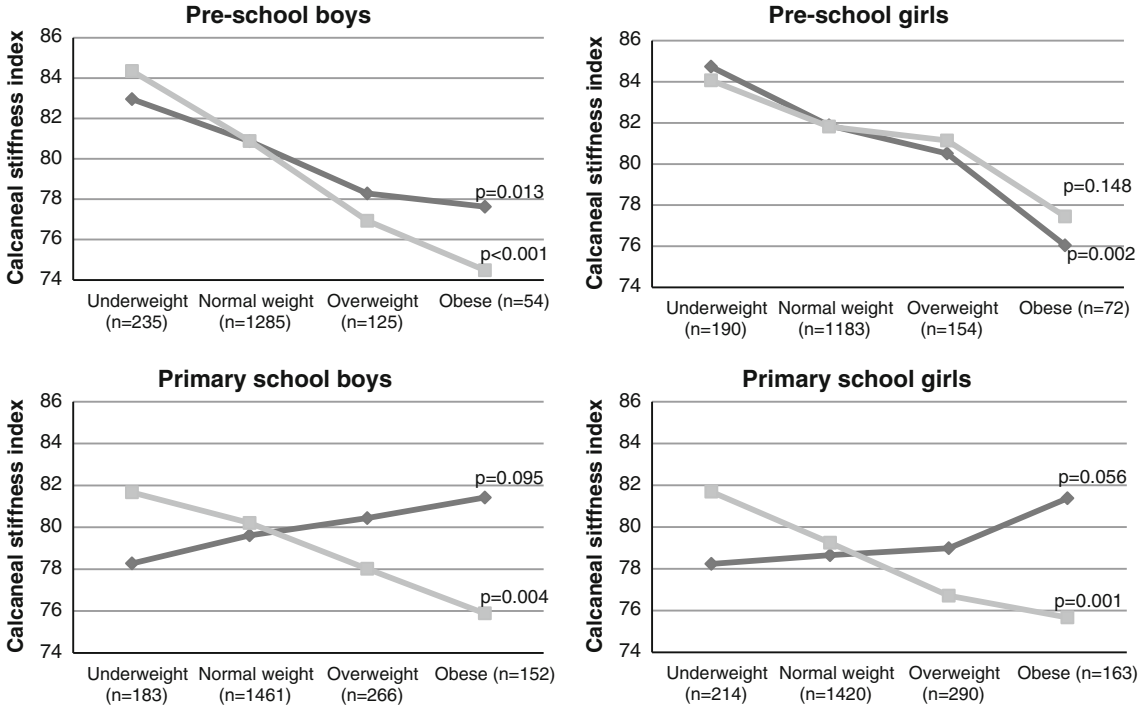

Fig. 2 Calcaneal stiffness index in relation to IOTF BMI categories in preschool and primary school boys and girls. *Lines between points* are only to facilitate interpretation. *Diamonds* adjusted for height and age, *squares* adjusted for height, age, and FFM

calcaneus. Next, Zadik et al. [34] investigated a sample of 1,085 healthy children and adolescents aged 0–18 and did not find a significant correlation between height, weight, and BMI, on the one hand, and SOS measured at the tibia and radius, on the other hand. In contrast to Zadik et al., Christoforidis et al. [35] found significant positive correlations between weight, height, and BMI and SOS measured at the tibia and radius in 1,549 healthy subjects between the ages of 4 and 18. In summary, the available data are inconsistent, also due to the use of different QUS devices. Moreover, it has to be mentioned that in very young skeletons under the age of 5 years, woven bone tissue is often found, whereas this is normally not the case in older children. Very little is known about the mechanical properties of this woven cortical bone tissue, but possibly the presence of this woven bone tissue can influence calcaneal SI in very young children.

QUS Results for Primary School Children

In primary school children, we found statistically significantly higher SI values in boys than in girls (79.7 in boys compared to 78.9 in girls). The findings of other studies are conflicting: some of them found no sex differences [13, 18, 19, 27, 33], while others reported a sex difference, sometimes limited to specific age groups [3, 21, 25, 47]. It is possible that the sex difference in QUS parameters is highly influenced by the sex difference in the onset of growth phases and the onset of maturity [3, 27]. Moreover, the positive associations found in these primary school children between SI, on the one hand, and age, weight, BMI, and waist circumference, on the other hand, are in accordance with other studies using QUS measurements in the same age group [13, 18, 19, 21, 23, 27, 47].

Limitations and Strengths

The strength of the current study is the availability of a large population-based sample of boys and girls aged 2–9 years from eight different European countries (north, south, east, and west distributed), from which QUS measurements as well as a large battery of anthropometric measurements were obtained. In this study analyses were limited to the cross-sectional data of the IDEFICS baseline survey.

The major weakness of the study was that the original BUA and SOS values measured by the QUS were not available. It is possible that other interesting correlations would have been found with the BUA and SOS parameters. Moreover, due to the lack of the BUA and SOS data, it is difficult to compare our results with the results of other studies reporting BUA and SOS values and not SI values. In addition, no data were available on the foot dimensions of the children, which might have been relevant as van den Bergh et al. [47] showed that foot length is an independent predictor for BUA and SOS in boys. However, by

correcting the analyses for height, this effect was possibly partly covered. Measurement of lean mass by DXA or Bodpod was logistically not feasible in a large-scale study such as IDEFICS where the fieldwork took place in schools. Therefore, measuring bioelectrical impedance and assessing FFM using a formula was the best available alternative. Finally, in this analysis physical activity and dietary patterns were not taken into account. Nevertheless, in subgroups of the IDEFICS study, physical activity levels of the children were assessed by accelerometers (in 3,978 of the 7,447 children, i.e., 53.4 %) and dietary habits were assessed by 24-h recall (in 4,545 of the 7,447 children, i.e., 61 %). In order not to impair the sample sizes for this article's objective, we did not include those parameters.

In spite of these limitations, this study provides to our knowledge the first data on calcaneal SI in European preschool children. Our results highlight that body fat is strongly associated with calcaneal SI in children. Based on the comparison between preschool and primary school children, it seemed that the relationship between different markers of body fat, on the one hand, and calcaneal SI, on the other hand, is opposite in preschool and primary school children. However, adjusting for FFM inversed the relation in primary school children. This means that body fat is negatively associated with calcaneal bone stiffness in children after adjustment for FFM. FFM may confound the association in primary school children but to a lesser degree in preschool children. Muscle mass may therefore be an important determinant of bone stiffness.

Health intervention programs need to consider that reducing body fat mass and keeping body weight within a normal range in children may positively influence bone stiffness in early lifetime and optimize peak bone mass.

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Appendix G

Influence of Birth Weight on Calcaneal Bone Stiffness in Belgian Preadolescent Children

Van den Bussche K, Michels N, Gracia-Marco L, **Herrmann D**, Eiben G, De Henauw S, Sioen I

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ORIGINAL RESEARCH

Influence of Birth Weight on Calcaneal Bone Stiffness in Belgian Preadolescent Children

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Abstract The aim of this study was to investigate the relation between birth weight and calcaneal bone stiffness in a large sample of Belgian, healthy, preadolescent children. Participants were 827 children (3.6–11.2 years, 51.6 % boys) from the Belgian cohort of the IDEFICS study. Birth weight was obtained using a parental questionnaire, and quantitative ultrasound (QUS) measurements were performed to determine calcaneal broadband ultrasound attenuation (BUA), speed of sound (SOS), and stiffness index (SI) using the Lunar Achilles device. Average birth weights were 3435.7 ± 512.0 g for boys and 3256.9 ± 471.1 g for girls. Average calcaneal QUS measurements were 89.6 ± 24.0 (23.3–153.9) dB/MHz for BUA, 1621.4 ± 49.6 (1516.3–1776.5) m/s for SOS, and 92.8 ± 15.6 (49.0–163.0) for SI. Birth weight was positively associated with BUA ($r = 0.13$, $p = 0.002$) and SOS ($r = –0.16$, $p < 0.001$). The associations remained after correcting for age and sex in multiple regression analyses but disappeared after correcting for anthropometric covariates. Our findings suggest that birth weight, as a rough proxy indicator for genetic and environmental influences during intrauterine life, is associated with BUA and SOS in preadolescent children and may therefore influence the risk of osteoporosis later in life. Further studies using QUS are needed to investigate the consistency of the results of this study.

Keywords Birth weight · Children · Calcaneal quantitative ultrasound · Bone stiffness

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Osteoporosis is one of the most widespread, costly, and debilitating diseases in Europe [1, 2]. The World Health Organization (WHO) defined osteoporosis as a progressive, systemic skeletal disease that is characterized by low bone mass and microarchitectural deterioration of bone tissue, with a consequent increase in bone fragility and in fracture risk [3]. This skeletal disease most often appears acutely in the form of a fracture at high age, but the origin can be found at much younger age, with two major factors determining the increased fracture risk: achieved peak bone mass and loss of bone [4, 5]. Peak bone mass is almost entirely achieved in the first two decades of life, and the amount is determined by heredity, calcium, and vitamin D intake through nutrition, hormones, physical activity, and other lifestyle factors [6–12]. Recent studies have investigated other early determinants of osteoporosis, of which birth weight is one. At this stage, several studies have shown associations between birth weight and adult bone mass [13–17]. It is uncertain whether that influence of birth weight is already visible in childhood. Several studies with varying sample sizes (from 64 to 6,876) have investigated birth weight and bone health in children between the ages of 6 and 10 years [18–24]. The study results are hard to compare and are not conclusive. Moreover, no literature is available for children at preschool age. Generally, no recent studies investigating the influence of birth weight on bone health in young Belgian children are available. To provide more in-depth knowledge on the relation between birth weight and bone health in young children, this study investigated a large sample of Belgian, healthy, preschool and primary school children. To determine the bone strength in these children, we used quantitative ultrasound (QUS), a radiation-free tool [25]. The QUS parameters speed of sound (SOS) and broadband ultrasound attenuation (BUA) are related to trabecular bone, the most metabolically active bone tissue, with a higher variation rate compared to cortical bone tissue. QUS can be measured at different sites, of which the calcaneus is the most popular since it consists almost entirely of trabecular bone [25, 26]. So far, the number of studies using QUS to investigate the relationship between birth weight and bone strength in pre- and primary school children is limited [24].

Therefore, the purpose of this research was to further investigate the influence of birth weight as an independent variable on bone strength assessed by calcaneal bone stiffness in a large sample of healthy children at prepubertal age.

Materials and Methods

Subjects

The subjects were participants of the Belgian cohort of the EU Sixth Framework Programme IDEFICS study (Identification and Prevention of Dietary- and Lifestyle-Induced Health Effects in Children and Infants, www.idefics.eu). The IDEFICS study is a unique longitudinal and multicenter study investigating factors that influence the health, growth, and development of European children, with emphasis on obesity and its comorbid conditions. The study was conducted in eight European countries and included two measurement periods: a baseline survey in 2007–2008 and a follow-up survey in 2009–2010. In this article, only data on the Belgian IDEFICS participants were used and the bone parameters were collected in 2009–2010. The participating Belgian children were residents from two regions in the northern Dutch-speaking part of Belgium: the city of Aalter (51°05′N, March–June 2010) and the city of Geraardsbergen (50°46′N, October 2009–February 2010). At baseline, one intervention and one control region, which were comparable with regard to their infrastructural, sociodemographic, and socioeconomic characteristics, were selected. Children were approached through school and kindergarten settings in both selected regions, which facilitated enrollment [27–30]. For the purpose of this analysis, data on 827 children aged 3–11 years (427 boys and 400 girls, mean age 7.7 ± 1.5 years) were available (227 from Geraardsbergen and 600 from Aalter), on which QUS measurements were performed, and data on birth weight were available. Twins or triplets were excluded since multiple birth influences birth weight. The study was conducted according to the guidelines laid down in the Helsinki Declaration of the World Medical Association. The project protocol was approved by the Ethical Committee of the Ghent University Hospital. Written informed consent was obtained from all parents of the participating children.

Measurements

Questionnaire

A self-administered parental questionnaire was used to obtain information on the following variables: sex of the child, birth date, birth length, and birth weight. The age of the child at time of examination was calculated using date of birth and date of examination.

Quantitative Ultrasound

QUS measurements were performed with a Lunar Achilles Insight (GE Healthcare, Milwaukee, WI). This portable device measures bone stiffness using ultrasound waves. The first outcome parameter, BUA, reflects the absorption of sound waves and is expressed as decibels per megahertz ($n = 596$). The second parameter, SOS, expresses the stiffness of a material by the ratio of the traversed distance to the transit time, in meters per second ($n = 600$). The

more complex the bone structure, the more sound waves will be absorbed. Stiffness index (SI) is a third, derived parameter ($n = 827$). SI is calculated by a linear combination of BUA and SOS: SI = (0.67 × BUA) + (0.28 × SOS) − 420 [31, 32]. The real-time image of the calcaneus and the region of interest ensures that the measurement is precise and was set to 18 mm diameter. During the entire study period one instrument was used, and daily calibration was done. Measurements were made according to the standard procedure provided by the manufacturer. The use of an adapter for the children's feet ensured the proper position of the calcaneus. The main heel bones (os calcis) of both feet were measured once, and the mean of the two measurements was calculated and used in the statistical analyses. The overall QUS measurement required about 10 minutes per child. When the child moved too much during the measurement, no result was given by the device and the measurement had to be redone.

Anthropometric Measurements and Body Composition

Anthropometric measurements were performed by two trained researchers. Height was measured with a standard clinical Seca 224 stadiometer (Seca, Hamburg, Germany) to the nearest 0.1 cm. Weight was determined with a standard balance (BC 420 SMA; Tanita, Amsterdam, The Netherlands) to the nearest 0.1 kg, without shoes and in light clothing. The two measurement instruments did not need further calibration or maintenance except for daily verification of the degree of horizontality. The Tanita balance (adapted to the small foot size of children) also measured leg-to-leg impedance (ohm). The Tyrrell formula was used to calculate the fat-free mass (FFM, in kilograms) based on this impedance value [33]. To take weight into account, %FFM was computed using the formula %FFM = (FFM/weight) × 100.

Body mass index (BMI) was computed according to the following formula: BMI = weight (kg)/height^2 (m^2) [34]. The z scores of each child's weight, height, and BMI were determined using the LMS method (with British reference population), which summarizes the distribution of these variables at each age by its median and coefficient of variation, plus a measure of skewness based on the Box–Cox power required to transform the data to normality [35]. Waist and hip circumferences were measured using a Seca 200 inelastic tape (precision 0.1 cm, range 0–150 cm), which did not need any calibration. The ratio of waist/hip circumference was calculated and used in the analyses. A skinfold caliper (Holtain, Crosswell, UK; range 0–40 mm) was used to measure skinfold thickness at the previously marked points. The calipers were calibrated every morning and additionally when dropped by means of a calibration block of 20 mm. Skinfold measurements were obtained at two sites (triceps and subscapular) according to the international standards for anthropometric assessment [36]. Skinfold thickness was measured twice at each site, and the mean of the two measurements was calculated. If measurements differed more than 2 mm, a third measurement was performed and the mean was calculated between the two closer values differing less than 2 mm. Additionally, the sum of the two skinfold thicknesses was computed and used as an indicator of the fat distribution in the upper limbs.

Statistical Analysis

After logarithmic transformation of tricipital skinfold thickness, subscapular skinfold thickness, the sum of the two, and the waist/hip ratio, all residuals showed a satisfactory pattern (normal distribution). Descriptive data by sex were examined with independent samples t tests (for normally distributed variables) and Mann–Whitney U-tests (for non-normally distributed variables). Since there was no difference observed in bone variables between sexes ($p > 0.05$), boys and girls were analyzed together. Pearson correlation coefficients were performed to define potential confounders in the association bone–birth weight. Stepwise multiple regression analysis was used to find the best models predicting the dependent variables BUA, SOS, and SI. Birth weight was included as an independent variable, and sex and age were included as confounders in all analyses since sex had an influence on birth weight and age, on anthropometric variables. Different variables were included as covariates in multiple models, separate for BUA, SOS, and SI. All statistical measurements were obtained using the PASW Statistics Program, version 20.0.0 (SPSS, Inc., Chicago, IL); and statistical results with $p < 0.05$ were considered statistically significant.

Results

Subject Characteristics

Information on early life factors, body composition, and current characteristics is summarized in Table 1. The mean birth weight, birth length, and mean %FFM were slightly higher in boys compared to girls ($p < 0.001$). Tricipital, subscapular, and the sum of both skinfold thicknesses were higher in girls compared to boys ($p < 0.001$). No sex differences in bone parameters were found.

Correlation Coefficients

Correlation analyses between birth weight and bone measurements are presented in Fig. 1. Significant correlations

Table 1 Descriptive characteristics of the studied children by sex: mean ± SD

	Boys ($n = 427$)	Girls ($n = 400$)	p (sex difference)
Early life factors			
Birth weight (g)	3435.7 ± 512.0	3256.9 ± 471.1	<0.001
Birth height (cm)	50.6 ± 2.3	49.7 ± 2.6	<0.001
Current subject characteristics			
Calcaneal BUA (dB/MHz)	91.4 ± 25.3	87.7 ± 22.6	0.066
Calcaneal SOS (m/s)	1621.2 ± 47.3	1621.6 ± 51.9	0.925
Calcaneal SI	93.7 ± 15.5	91.9 ± 15.6	0.097
Age (years)	7.8 ± 1.5	7.7 ± 1.6	0.244
Height z score	0.4 ± 1.0	0.3 ± 1.1	0.203
Height (cm)	128.6 ± 10.7	127.3 ± 10.5	0.087
Weight z score	0.2 ± 1.0	0.1 ± 1.1	0.765
Weight (kg)	26.5 ± 6.0	26.3 ± 6.5	0.555
BMI z score	−0.1 ± 1.0	−0.1 ± 1.2	0.684
Waist/hip ratio[a]	0.9–0.05	0.85–0.06	0.066
Tricipital skinfold thickness (mm)[a]	8.75–3.5	10.9–4.9	<0.001
Subscapular skinfold thickness (mm)[a]	5.4–1.5	6.4–2.9	<0.001
Sum skinfolds (mm)[a]	14.1–4.4	17.2–7.7	<0.001
Fat-free mass (%)	75.9 ± 5.4	69.7 ± 10.1	<0.001

SI stiffness index, *BUA* broadband ultrasound attenuation, *SOS* speed of sound, *IOTF* International Obesity Task Force

[a] Mann–Whitney *U*-test. Median–interquartile range

were found between birth weight and SOS ($r = –0.16$, $p < 0.001$) as well as between birth weight and BUA ($r = 0.13$, $p = 0.002$). No association was observed between birth weight and SI. Table 2 shows the correlation coefficients between the calcaneal bone parameters (BUA, SOS, and SI), age, and anthropometric variables. BUA and SOS were significantly associated with age and all the anthropometric variables (all $p < 0.05$), except for the association between SOS and %FFM ($p = 0.316$). SI was significantly correlated with height, weight, FFM, and %FFM (all $p < 0.05$). Generally, the skinfolds and BMI z score had a weak association with the bone parameters, in contrast to the variables height, weight, and waist z score. FFM was strongly associated with BUA, SOS, and SI (all $p < 0.001$); but that association weakened when using the %FFM. The anthropometric variables height, weight, and FFM were further analyzed in stepwise multiple regression analysis due to high correlation coefficients. The variables birth length, BMI z score, waist z score, waist/hip ratio, and skinfold thickness were not retained because of low correlation coefficients.

Stepwise Multiple Regression

Multiple regression analyses were performed to explore independent variables (including birth weight) influencing BUA, SOS, and the calculated parameter SI. Table 3 shows the association between calcaneal BUA and birth weight. Model A shows raw data, model B = model A + sex and age (confounders), model C = model B + weight z score, model D = model C + %FFM, and model E = model C + height z score. Height z score and %FFM could not be together in the model due to multicollinearity, a high correlation between both covariates. Birth weight retained a positive association with BUA after controlling for age and sex (model B) but showed no significant associations after controlling for anthropometric variables (models C, D, and E). Sex was not associated with BUA in any model. Age influenced BUA in four models except in model C, where weight z score was added. Finally, weight z score, %FFM, and height z score were significantly associated with BUA. The adjusted R^2 was low for model A ($R^2 = 0.015$) but higher after controlling in the other models. Table 4 shows the association between calcaneal SOS and birth weight. Model A shows raw data, model B = model A + sex and age (confounders), model C = model B + weight z score, and model D = model C + height z score. Birth weight retained a negative association with SOS, also after controlling for age and sex (model B), but showed no significant associations after adjusting for weight z score (model C) and height z score (model D). Age, weight z score, and height z score were independently associated with SOS. As was the case for calcaneal BUA, sex had no influence on calcaneal SOS. The unadjusted model A explained less of the variation in SOS than the adjusted models. Table 5 shows the association between calcaneal SI and birth weight. Model A shows raw data, model B = model A + sex and age (confounders), and model C = model B + %FFM. In line with the insignificant univariate correlation between birth weight and SI, birth weight did not predict SI in any of the regression models. Age was the only predictor of SI ($p < 0.001$). The variable %FFM did not show significant associations with SI. The adjusted R^2 was very low in all models.

Discussion

This study investigated the relationship between birth weight and bone strength, assessed as calcaneal BUA, SOS, and SI measured by QUS, in 827 healthy children,

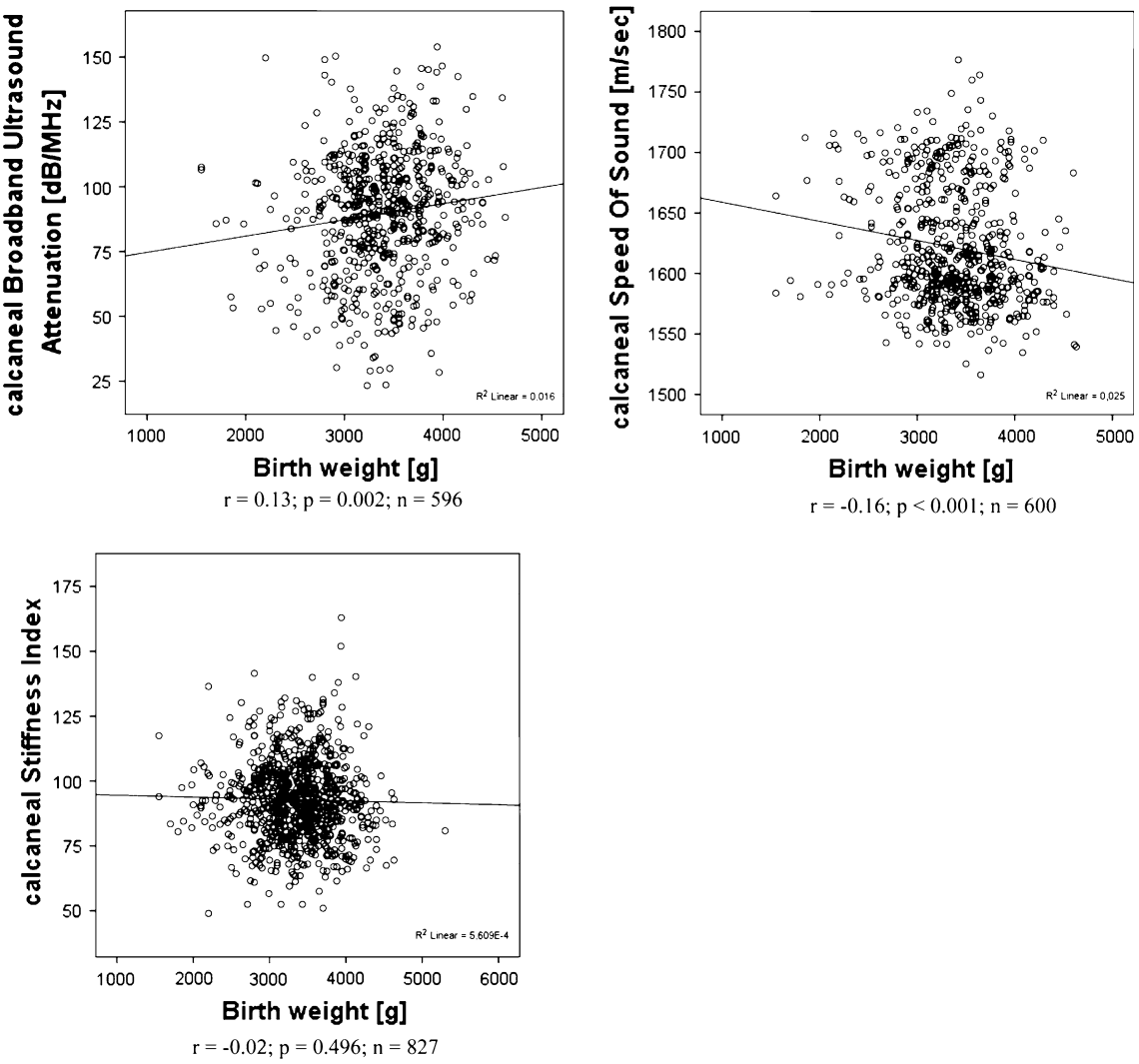

Fig. 1 Scatterplots and regression lines of the relation between birth weight and calcaneal broadband ultrasound attenuation, speed of sound, and stiffness index (each *hollow circle* is a case; below each scatterplot the Pearson correlation coefficient as well as the *p* value are given)

aged 3–11 years. The main findings were that birth weight was significantly positively correlated with BUA and significantly negatively correlated with SOS even after correction for age and sex in multiple regression analyses. When adjusting for other covariates such as weight and height, the association with birth weight did not persist. No significant correlation was found between birth weight and SI. Sex had no influence on bone strength, and age was positively related with BUA and SI and negatively related with SOS.

Inconsistent results exist on the effect of birth weight on bone strength in preadolescent populations. Both Liao et al. [37] and Micklesfield et al. [24] investigated the influence of birth weight on bone properties using QUS measurements at the tibia and calcaneus, respectively. Liao et al. [37] concluded that birth weight had a positive influence on bone strength at the age of 3 months in 542 Chinese children, while no significant correlations were found between birth weight and BUA and SOS in 109 South African children between the ages of 7 and 9 years [24]. The first study is not comparable with our study sample due to differences in nationality and age of the population. The second study examined the same bone site as in our study, but the mixed ancestral origin, smaller sample size, or use of the nondominant calcaneus could possibly explain the lack of evidence supporting this association [24].

Four studies investigated the influence of birth weight, with correlation analyses, on bone properties using total-body dual-energy X-ray absorptiometry (DXA) measurements [18–21]. Ganpule et al. [18] assessed total-body bone mass density (BMD) in 698 Indian children aged 6 years and concluded that birth weight was positively correlated with increased total-body BMD. Macdonald-Wallis et al. [20] assessed different bone properties at the spine and total

Table 2 Results of Pearson's correlations between bone parameters and anthropometric variables

	BUA (dB/MHz) ($n = 596$)	SOS (m/s) ($n = 600$)	SI ($n = 827$)
Age (years)	0.49***	–0.45***	0.17***
Birth length (cm)	0.16***	–0.15***	0.00
Height z score	0.30***	–0.36***	0.02
Height (cm)	0.57***	–0.57***	0.15***
Weight z score	0.29***	–0.38***	0.01
Weight (kg)	0.53***	–0.57***	0.11***
BMI z score	0.16***	–0.26***	–0.01
Waist z score	0.24***	–0.34***	0.01
Waist/hip ratio (log)	–0.17***	0.16***	–0.01
Tricipital skinfold thickness (log)	0.12**	–0.25***	–0.06
Subscapular skinfold thickness (log)	0.15***	–0.26***	–0.03
Sum skinfolds (log)	0.14***	–0.27***	–0.05
Fat-free mass (kg)	0.57***	–0.55***	0.16***
Fat-free mass (%)	0.08*	0.04	0.09**

(log) log-transformed variable

* $p < 0.050$, ** $p < 0.010$, *** $p \leq 0.001$

body in 6,877 9-year-old children and found similar positive correlations with birth weight. Micklesfield et al. [21] examined 64 children between 7 and 9 years old with DXA and did not find any positive correlations with birth weight, though the dissimilarity in sample size and method of measurement could explain the different results. Finally, Jones and Dwyer [19] investigated BMD of the lumbar spine and femoral neck in 330 Australian children at the age of 8 and concluded that birth weight has an influence on femoral neck BMD but not on lumbar spine BMD.

In summary, six studies examined correlations between birth weight and different bone parameters in prepubertal children, without taking potential confounders into account. Half of the studies drew similar conclusions as we did. The other half did not find any relationship between birth weight and bone health which could be explained by differences in study population and design.

Only four studies investigating the relation between birth weight and bone health in preadolescent children considered also the role of potential covariates [19, 22, 23, 38]. In these studies, bone properties were measured using DXA: at the lumbar spine and femoral neck by Jones and Dwyer [19]; the total body by Steer and Tobias [22]; the lumbar spine, femoral neck, and total body by Vidulich et al. [23]; and total body and lumbar spine by Ay et al. [38]. Ay et al. [38] examined 252 Dutch children at the age of 6 months and found an influence of birth weight on BMD and bone mineral content (BMC) after correcting for sex, gestational age, and current age. Unlike our study, Ay et al. [38] did not add other potential covariates such as anthropometric variables in the regression model. Steer and Tobias [22] found similar results in 109 9-year-old children when only correcting for sex, age, and gestational age. However, after additionally correcting for parental weight and height in a second model and the child's weight and height in a third model, this association decreased but was still significant with the exception of BMC and bone area in the third model. Similar effects were found in 330 Australian 8-year-old children: a relationship between birth weight and BMD when correcting for sex and growth variables and even when additionally correcting for breast-feeding, maternal smoking during pregnancy, calcium intake, sunlight exposure, and sports participation [19]. However, the association disappeared after additionally correcting for maternal BMD. Also, in 476 10-year-old

Table 3 Results of stepwise multiple regression analyses using calcaneal BUA ($n = 596$) as a dependent variable and various other variables as independent variables

Variables	Model A ($R^2 = 0.015$)[a]		Model B ($R^2 = 0.252$)		Model C ($R^2 = 0.338$)		Model D ($R^2 = 0.351$)		Model E ($R^2 = 0.346$)	
	B[b]	p	B	p	B	p	B	p	B	p
Birth weight (g)	0.006	**0.002**	0.006	**0.001**	0.002	0.230	0.002	0.339	0.002	0.994
Age (years)			7.422	**<0.001**	7.616	0.510	7.442	**<0.001**	7.566	**<0.001**
Sex			–1.565	0.367	–2.165	0.185	–0.013	0.994	–1.824	0.262
Weight z score					7.166	**<0.001**	8.255	**<0.001**	4.534	**<0.001**
FFM (%)							0.374	**<0.001**		
Height z score									3.537	**0.003**

Model A shows raw data, model B = model A + sex and age (confounders), model C = model B + weight z score, model D = model C + %FFM, and model E = model C + height z score

FFM fat-free mass (lean mass), *BUA* broadband ultrasound attenuation

p values <0.05 are indicated in bold

[a] Adjusted R^2

[b] Unstandardized regression coefficients

Table 4 Results of stepwise multiple regression analyses using calcaneal SOS ($n = 600$) as a dependent variable and various other variables as independent variables

Variables	Model A ($R^2 = 0.023$)[a]		Model B ($R^2 = 0.224$)		Model C ($R^2 = 0.359$)		Model D ($R^2 = 0.366$)	
	B[b]	*p*	B	*p*	B	*p*	B	*p*
Birth weight (g)	–0.016	**<0.001**	–0.016	**<0.001**	–0.006	0.073	–0.005	0.117
Age (years)			–14.217	**<0.001**	–14.699	**<0.001**	–14.607	**<0.001**
Sex			–4.132	0.254	–2.395	0.468	–3.068	0.351
Weight *z* score					–18.488	**<0.001**	–13.603	**<0.001**
Height *z* score							–6.577	**0.007**

Model A shows raw data, model B = model A + sex and age (confounders), model C = model B + weight *z* score, model D = model C + height *z* score

SOS speed of sound

p values <0.05 are indicated in bold

[a] Adjusted R^2

[b] Unstandardized regression coefficients

Table 5 Results of stepwise multiple regression analyses using calcaneal SI ($n = 827$) as a dependent variable and various other variables as independent variables

Variables	Model A ($R^2 = -0.001$)[a]		Model B ($R^2 = 0.029$)		Model C ($R^2 = 0.032$)	
	B[b]	*p*	B	*p*	B	*p*
Birth weight (g)	–0.001	0.496	–0.001	0.344	–0.001	0.391
Age (years)			1.694	**<0.001**	1.649	**<0.001**
Sex			–1.770	0.104	–0.935	0.423
FFM (%)					0.123	0.067

Model A shows raw data, model B = model A + sex and age (confounders), and model C = model B + %FFM

FFM fat-free mass (lean mass), *SI* stiffness index

p values <0.05 are indicated in bold

[a] Adjusted R^2

[b] Unstandardized regression coefficients

South African children with mixed ancestral origin no association between birth weight and bone health was found after adjusting for bone age, sex, race, socioeconomic status, current height, and weight [23]. Taken together, our research—having the advantage that radiation-free QUS was used—gave similar results as literature using bone health parameters assessed by DXA: an association between birth weight and bone health parameters after correcting for age and sex but less or no association after correcting for additional confounders.

Analyzing the relationship between birth weight and adult bone mass, an important difference should be indicated. In adults, birth weight is more associated with BMC compared to BMD [13, 16]. Baird et al. [13] put forward that the absence of an association with areal or volumetric BMD could suggest that this highly conserved aspect of bone structure is largely determined by other postnatal factors (e.g., pubertal timing and physical activity in childhood) or by fixed genetic variation.

Strengths and Limitations of the Study

The strength of this study is the availability of a large representative population-based sample of both boys and girls from Belgium of preschool and primary school age. Compared to other studies investigating birth weight and bone health in childhood, this cohort has a large sample from a previously unexplored age group (3–11 years). Data on early life factors, QUS measurements, and a large battery of anthropometric measurements were complete for all 827 children. Consequently, the analyses could be corrected for potential confounders (age, sex, and anthropometric variables). To ensure quality, trained researchers performed the QUS and anthropometric measurements. For the QUS measurement both feet (os calcis) were measured, which has the advantage of showing bone metabolic changes first since it consists of 90 % trabecular bone and has a high turnover rate [39]. The average of the parameters measured at the left foot and at the right foot was

calculated. In the literature, different approaches can be found: some measure the right foot [40], the left foot [41, 42], twice the dominant leg [43], or both but choose the left foot when there is no difference in results [44]. Taking two feet into account could increase the accuracy.

Nevertheless, this study has some drawbacks as well. The first is the absence of an exact gestational age per child as this could be an important potential confounder [18, 20, 37, 38]. A second limitation is the difference between the number of primary school children ($n = 685$) and preschool children ($n = 142$). Therefore, analyses were not divided by age groups, but age was added as a confounder in all analyses. Thirdly, birth weight was obtained through a parental questionnaire and not by direct measurements or an existing register. Finally, a few remarks on the bone measurements with the QUS method. First, with this method inaccurate measurements can occur due to difficult positioning and immobilization of the small feet in children. Therefore, trained and only a limited number of researchers were used to obtain the QUS data. Second, the original BUA and SOS values measured by QUS were not available for all participants in BUA ($n = 596$) and SOS ($n = 600$) compared to SI ($n = 827$), due to different registration settings of the measuring device. Finally, QUS is a practical device but is not yet accepted as a standard measurement method in children [45]. Nevertheless, using radiation-free QUS could increase the participation ratio in a child population, which is necessary in this kind of research.

Conclusion

The present findings support the hypothesis that birth weight, a proxy indicator of genetic intrauterine environmental factors, may have a long-term impact on bone health and may be associated with the risk of osteoporosis much later in life. As a result, this study points toward the importance of a normal birth weight even in healthy children (including preschool children). Public health strategies should insist also on the importance of a normal birth weight as a basis for prevention of chronic diseases later in life. Guaranteeing optimal birth weight helps in attaining maximal peak bone mass and could even prevent osteoporosis later in life. Further research could investigate whether the findings of this study are consistent in large study samples when using the QUS method as well.

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