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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 standardised 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 (<2.5 mmol/l) or lower 25OHD (<43.0 nmol/l).

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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 exercises (WBEs) 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.

Methods

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), 16,228 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 osteopaenia, 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.

Fig. 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 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

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