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Bone mass development is sensitive to insulin resistance in adolescent boys

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ABSTRACT

Purpose: Insulin resistance may exert a negative influence on bone mass in childhood and adolescence. The objective was to assess the association between insulin resistance and total body less head (TBLH) bone mineral content (BMC) and to investigate whether body composition, physical activity or osteocalcin levels may influence this association.

Methods: A longitudinal study with follow-up over more than 6 years was performed and included 562 apparently healthy participants with a mean age of 9.6 years at baseline.

Participants underwent DXA scanning at baseline. At the two follow-ups, participants had performed another DXA scanning, had blood samples taken for fasting insulin, glucose and osteocalcin and had physical activity measured with an accelerometer. HOMA-IR was calculated as an index of insulin resistance.

Results: HOMA-IR was negatively associated with TBLH BMC in boys at follow-ups ($\beta = -31.4$, $p < 0.001$) after adjustment for maturity, height, bone area, and baseline level of TBLH BMC. The negative association remained almost unchanged after further adjustments for body composition and physical activity. No association between HOMA-IR and TBLH BMC was found in girls.

Conclusion: Insulin resistance may be detrimental for bone development through puberty in boys independent of body composition and the level of physical activity.

1. Introduction

Bone mass increases during childhood and adolescence until peak bone mass is reached [1]. As the risk of osteoporosis later in life may be reduced by increasing peak bone mass [1,2], it is important to identify factors that influence peak bone mass.

Insulin itself may have osteogenic effects [3], and cross-sectional studies report a positive association between bone mass and fasting insulin in different age groups [4–6]. However, this association disappears or changes direction after adjustment for body composition. In line with this data Torres-Costoso et al. [7] suggested an influence from body composition on the association between insulin resistance and bone mass. Body composition is known to be associated with bone mass level in childhood and adolescence, where lean mass may contribute more to the variance in bone mass than fat mass [8,9].

The association between bone mass and fasting insulin or insulin

Abbreviations: TBLH, total body less head; BMC, bone mineral content; DXA, Dual energy X-ray absorptiometry; %FM, whole body percent fat mass; HOMA-IR, homeostasis model assessment of insulin resistance; APHV, age at peak height velocity; PA, physical activity; MVPA, moderate to vigorous physical activity; VPA, vigorous physical activity

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resistance (as expressed by the homeostasis model assessment of insulin resistance, HOMA-IR) may be sex-specific as suggested in some cross-sectional studies [5,10,11]. Studies with longitudinal data in children are few, but Dalskov et al. [12] found no association between insulin and bone mass over a time period of six months.

Physical activity (PA) is known to be positively associated with bone mass in childhood and adolescence [13] and may also alter insulin resistance in children [14,15]. However, a potential interaction between insulin resistance and PA on bone mass has not been fully elucidated.

Osteocalcin (OC) is a bone specific non-collagen protein synthesised by osteoblasts and is a sensitive marker of bone formation [16]. After synthesis, OC is carboxylated and then mainly incorporated into bone matrix. OC is also thought to play a role in glucose metabolism [17,18] and may act as a hormone to increase insulin sensitivity [19]. The evidence for an association between OC and glucose metabolism in children is sparse, however; and Giudici et al. [20] did not observe an association between osteocalcin and HOMA-IR in healthy children. The exact mechanisms of OC functions are unclear, but it is thought to be the undercarboxylated OC (ucOC) that represents the active part acting as a hormone [18,21]. Both fully carboxylated OC and ucOC are measurable in the circulation, and no clear conclusions can be made.

The objectives of the present study were 1) to examine the association between insulin resistance and bone mass in healthy children and adolescents, and 2) to investigate whether body composition, physical activity or osteocalcin may influence such an association.

2. Methods

2.1. Ethical considerations

Children and parents received written information prior to study start as well as verbal information at school meetings. Written informed consent was provided by parents before entering the study. Participation was voluntary for all children, and consent could be withdrawn at any time. The CHAMPS-study DK was approved by the Regional Scientific Ethical Committee of Southern Denmark (Project ID:S2008-0047, S-20140105). The study was accepted by the Danish Data Protection Agency.

2.2. Study design and participants

The Childhood Health Activity and Motor Performance School Study, Denmark (CHAMPS-study DK) is a longitudinal school-based observational study conducted from 2008 to 2015, described in detail elsewhere [22]. Children aged 6–11 years at baseline from 10 public schools in the Danish municipality of Svendborg were invited to participate. The present sub-study included the oldest part of the cohort attending 2nd – 4th grade at baseline (7.7–12.0 years old). Participants with diabetes or bone disease were excluded from this sub-study. Participants with at least one follow-up examination (2-years follow-up and the last follow-up after > 6 years) in addition to the baseline examination were included in statistical analyses.

2.3. Dual energy X-ray absorptiometry

Dual energy X-ray absorptiometry (DXA) scans were performed at three time-points (autumn/winter 2008/2009, autumn winter 2010/2011 and spring 2015) using a GE Lunar Prodigy with enCORE software (version 15; GE Medical Systems, Madison, WI, USA). All scans were conducted using the same DXA scanner at Hans Christian Andersen Children's Hospital, Odense University Hospital, Denmark, with three operators performing all scans and two operators (MH and MSR) analysing all data.

Participants were scanned in a supine position wearing undergarments, stockings and a thin T-shirt and lying under a thin blanket. The scan depth was automatically altered according to age, height and weight of the child. The scanner had a height limit of 195 cm, and participants taller than this limit were excluded before DXA scanning.

The scanner was tested for reproducibility and precision every morning using a standard phantom in accordance with the recommendations from the manufacturer. The scanner had a coefficient of variation (CV%) for the phantom areal bone mineral density (aBMD) of 0.27–0.33% during the whole study period.

Total body less head (TBLH) values were used to obtain bone mineral content (BMC) and bone area. Also whole body percent fat mass (%FM) was obtained by the DXA scan and used in combination with weight as a measure for body composition.

2.4. Anthropometry and maturational development

Before the DXA scan, participants had height and weight measured. Height was measured with a portable stadiometer to the nearest 0.1 cm (SECA 213), and weight was measured to the nearest 0.1 kg on an electronic scale (SECA 861) (Both SECA GmbH & Co. KG, Hamburg, Germany).

Maturational status (maturity) was defined as number of years from age at peak height velocity (APHV) at the examination. Maturity was calculated based on the child's age and height according to the equations described by Moore et al. [23]. The height measurement performed nearest to the time for APHV for that child was used.

2.4.1. WHO Z-scores

Body mass index (BMI; defined as “weight in kg/height in m squared”) was calculated and used to further calculate age- and sex-adjusted World Health Organisation (WHO) reference *Z*-scores for BMI using a software package from WHO [24]. These Z-scores were used to define overweight (WHO-BMI-Z-score > 1) and obesity (WHO-BMI-Z-score > 2) [25].

2.5. Laboratory analyses

Blood samples were obtained at the participants schools following an overnight fast (at least 8 h was requested). Fasting status was verbally confirmed by the child. The samples were taken between 08.00 and 10.00 a.m. by trained biomedical laboratory technicians and were processed and centrally stored at −80 °C within 4 h.

Samples were analysed for glucose and insulin in an ISO 9001:2008 certified routine laboratory associated with the University of Vienna, Austria. Glucose levels were assessed on a Cobas C System (Roche Diagnostics GmbH, Mannheim, Germany), while insulin levels were assessed on an Access Immunoassay System (Beckman Coulter, Inc. Brea, CA, USA) using the respective kits.

Osteocalcin measurements were performed on the IDS-iSYS automated system using the chemiluminescence immunoassay IDS-iSYS N-MID®Osteocalcin (Immunodiagnostic Systems, Tyne & Wear, UK) at the Department of Clinical Biochemistry, Glostrup, Rigshospitalet, Denmark, which is ISO 15189 certified.

Intermediate precision (CV%) ranged from 0.5 to 0.8% for glucose, from 3.1 to 5.6% for insulin and from 3.0% to 3.6% for osteocalcin.

HOMA-IR [26] was calculated as (fasting insulin in μIU/ml * fasting glucose in mmol/l/22.5) and was used as a surrogate marker of insulin resistance.

2.6. Physical activity

PA was assessed objectively by GT3X and GT3X+ accelerometers (ActiGraph, Pensacola, Florida, USA). Measurements were performed in autumn 2010 and spring 2015.

Accelerometers were programmed to start recording the day after distribution and were collected after at least 7 days of wear. Participants were asked to wear the device on the right hip during all

waking hours and to remove it only for water-based activities. All consecutive strings of zero counts spanning 30 min or more were interpreted as "accelerometer not worn", and these strings were deleted from the data. The accelerometers stored data every 2 s (GT3X) and in 30 Hz (GT3X+). Recordings were collapsed into a 10-second epoch for analyses according to previous studies from the CHAMPS-study DK [27,28]. Participants with a minimum of 3 days recording with at least 10 h valid recording per day were included in the analyses. The data output were analysed using customized software (Propero version 1.7.4, University of Southern Denmark, Odense, Denmark). Accelerometer recordings were coded as moderate to vigorous physical activity (MVPA; ≥ 2296 counts per minute) or vigorous physical activity (VPA; ≥ 4012 counts per minute) according to Evenson et al. [29,30]. The percentage of wear-time spent engaging in MVPA and VPA was used in the analyses.

2.7. Statistical analyses

All statistical analyses were performed using STATA/IC 14.2 (StataCorp Lp, College Station, TX, USA). p values < 0.05 were considered statistically significant.

Differences between sexes were tested with unpaired *t*-tests.

2.7.1. Linear mixed-effect models

Linear mixed-effect models were used to analyse factors associated with TBLH BMC at the follow-ups. As a high degree of collinearity was found between age and maturity, only maturity was used in the models.

The basic model (model 1) was adjusted for baseline level of TBLH BMC and for sex, maturity, size (height and bone area) and the independent variable of interest (HOMA-IR) at follow-up.

An interaction term between HOMA-IR and sex was afterwards added to the model and if this was statistically significant, the analyses were stratified by sex. As %FM may also influence BMC differently in boys and girls, a sex-by-%FM interaction term was included in the models adjusting for body composition. The interaction term was omitted if non-significant.

The models incorporated school classes and participant identification as random effects.

After fitting the basic model, new models were built that included additional explanatory variables of body composition (weight and % FM), MVPA, VPA and OC.

3. Results

3.1. Participant characteristics

A total of 562 participants (277 boys) were included, with 1256 DXA scans (562 at baseline, 535 at 2-year follow-up, and 159 at the last follow-up; 132 participants had all 3 measurements) and follow-up data on fasting HOMA-IR (Fig. 1). A further 33 participants had missing PA data and were excluded from models adjusting for PA.

Mean age at baseline was 9.6 years (range 7.7 to 12.0 years), and 83.6% of baseline BMI measurements were within normal limits for BMI according to the WHO definition (16.4% were overweight, and of these 3.4% were obese).

At follow-ups, fasting glucose ranged from 3.5 to 6.4 mmol/l and fasting insulin from 0.9 to 23.41 μIU/ml (interquartile range for fasting insulin: 3.3 to 6.6 μIU/ml).

Further characteristics are presented in Table 1.

3.2. Associations with TBLH BMC

HOMA-IR was inversely associated with TBLH BMC in children aged 10–17 years at follow-ups after adjustment for maturity, sex, size (height and bone area) and baseline TBLH BMC ($\beta = -21.8$, $p < 0.001$) (Table 2). A significant interaction between HOMA-IR and sex was found ($\beta_{\text{HOMA-by-girl}} = 27.2$, $p < 0.05$), and further analyses were stratified by sex.

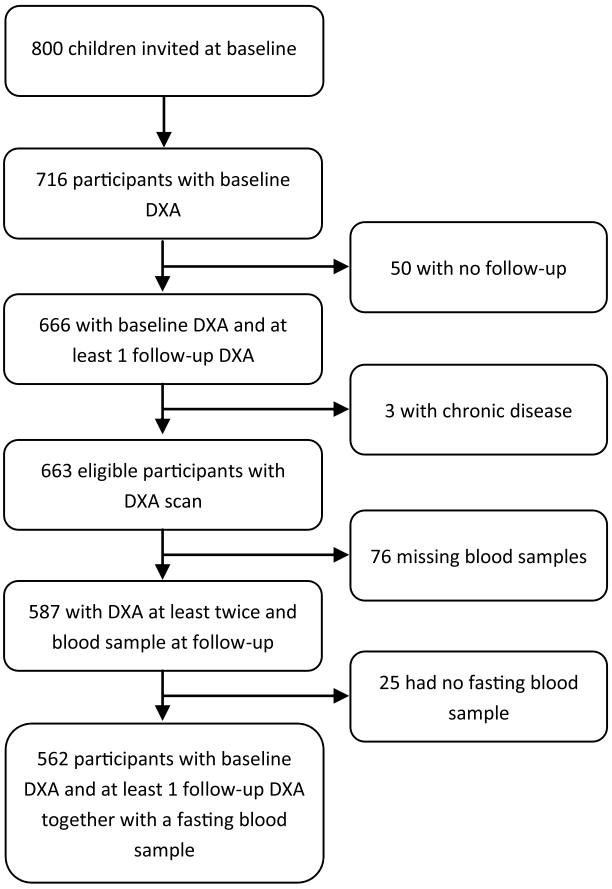

Fig. 1. Participant inclusion.
Flowchart of the inclusion of participants.

The sex difference was confirmed in the stratified analyses, where an inverse association between HOMA-IR and TBLH BMC was found in boys ($\beta = -31.4$, $p < 0.001$), but no association was found in girls in any of the models (Table 3).

Additional adjustment for body composition (weight and %FM), MVPA, VPA or OC yielded only minor changes in the magnitude of the association between HOMA-IR and TBLH BMC in boys (Table 3).

Fig. 2 shows the sex differences in TBLH BMC dependence on HOMA-IR and the amount of time spent engaging in VPA.

MVPA and VPA were positively associated with TBLH BMC in both sexes, showing no interaction with HOMA-IR. Body composition (weight and %FM) also showed significant associations with TBLH BMC in the same directions for boys and girls. While %FM was negatively associated with TBLH BMC, weight was positively associated with TBLH BMC.

4. Discussion

We found a highly significant inverse association between TBLH BMC and HOMA-IR in boys, whereby boys with higher HOMA-IR had on average lower TBLH BMC. The association was virtually unchanged after further adjustment for body composition, physical activity or osteocalcin. No association between HOMA-IR and TBLH BMC was observed in girls, suggesting that insulin resistance influences bone mass in a sex-dependent manner in children aged 10–17 years.

Our findings are thus in line with results from previous cross-sectional studies [5,10,11] that found similar sex differences in the association between HOMA-IR and bone mass. The participants in this study

Table 1
Participant characteristics.

Key variables		2008[a] Boys n = 277, girls n = 285		2010[a] Boys n = 265, girls n = 270		2015[a] Boys n = 81, girls n = 78	
		Mean (SD)	p-Value[b]	Mean (SD)	p-Value[b]	Mean (SD)	p-Value[b]
Age (years)	Boys	9.6 (0.9)	0.50	11.5 (0.9)	0.78	15.5 (0.7)	0.76
	Girls	9.6 (0.9)		11.5 (0.9)		15.5 (0.8)	
Height (cm)	Boys	140.3 (7.8)	0.003	151.4 (9.0)	0.62	176.3 (7.8)	< 0.001
	Girls	138.4 (7.1)		151.1 (8.2)		167.3 (5.9)	
Weight (kg)	Boys	33.0 (6.6)	0.13	40.8 (9.0)	0.90	61.5 (9.8)	0.005
	Girls	32.2 (6.1)		40.9 (8.3)		57.5 (8.3)	
BMI (kg/m^2)	Boys	16.6 (2.2)	0.77	17.6 (2.5)	0.50	19.7 (2.1)	0.03
	Girls	16.7 (2.1)		17.8 (2.5)		20.5 (2.5)	
BMC (g)	Boys	890.8 (193.5)	0.004	1192.9 (286.7)	0.97	2190.1 (423.3)	< 0.001
	Girls	843.4 (197.1)		1193.8 (304.5)		1945.5 (345.3)	
aBMD (g/cm^2)	Boys	0.77 (0.05)	0.05	0.84 (0.07)	0.51	1.05 (0.10)	0.002
	Girls	0.76 (0.06)		0.84 (0.08)		1.01 (0.08)	
BA (cm^2)	Boys	1151.7 (177.4)	0.001	1409.7 (233.2)	0.62	2068.2 (254.4)	< 0.001
	Girls	1102.4 (174.8)		1399.7 (232.2)		1920.3 (218.0)	
HOMA-IR	Boys	0.8 (0.4)	0.003	1.0 (0.5)	< 0.001	1.3 (0.8)	0.037
	Girls	0.9 (0.7)		1.2 (0.6)		1.5 (0.7)	
OC (μg/l)	Boys	122.4 (34.7)	< 0.001	152.9 (49.7)	< 0.001	152.2 (62.6)	< 0.001
	Girls	144.6 (41.9)		174.4 (47.5)		74.5 (48.1)	
%FM	Boys	17.5 (7.6)	< 0.001	19.4 (8.0)	< 0.001	13.1 (6.4)	< 0.001
	Girls	23.2 (7.3)		24.5 (7.0)		27.3 (6.5)	
Years from PHV	Boys	−3.7 (0.9)	< 0.001	−1.8 (0.9)	< 0.001	2.1 (0.8)	< 0.001
	Girls	−2.3 (0.9)		−0.3 (0.9)		3.6 (0.8)	

Physical activity		2010 boys n = 238, girls n = 261		2015 boys n = 70, girls n = 73	
Days included	Boys	5.9 (1.2)	0.44	6.5 (1.8)	0.95
	Girls	6.0 (1.2)		6.5 (2.0)	
Hours pr. day	Boys	13.6 (0.8)	0.76	14.1 (0.9)	0.94
	Girls	13.6 (0.8)		14.1 (0.9)	
MVPA (%)	Boys	9.4 (3.1)	< 0.001	6.8 (2.6)	0.001
	Girls	6.7 (2.0)		5.5 (2.1)	
VPA (%)	Boys	3.7 (1.7)	< 0.001	3.1 (1.6)	< 0.001
	Girls	2.5 (1.1)		2.2 (1.4)	

Abbreviations: SD, Standard deviation; BMI, body mass index (weight/height^2); BMC, total body less head bone mineral content; aBMD, total body less head areal bone mineral density; BA, total body less head bone area; HOMA-IR, homeostasis model assessment of insulin resistance; OC, Osteocalcin (μg/l); %FM, whole body percent fat mass; PHV, peak height velocity; Days included, number of days with valid accelerometer data; Hours pr. day, hours per day with valid accelerometer data; MVPA, % time spent engaging in moderate to vigorous physical activity; VPA, % time spent engaging in vigorous physical activity.

[a] 2008: autumn/winter 2008–2009, 2010: autumn/winter 2010–2011, 2015: spring 2015.

[b] Differences between sexes were tested with unpaired *t*-test.

were apparently healthy children and adolescents without diabetes, and thus a higher HOMA-IR will mainly be driven by higher insulin levels as the range of glucose levels was relatively narrow. With increasing insulin resistance, the tissues become less sensitive to insulin and the anabolic effects from insulin diminish. This may partly explain the negative association between HOMA-IR and TBLH BMC in adolescent children.

We are unable to explain the sexually dimorphic associations. However, it can be speculated whether the differences in sex-hormones between boys and girls may influence the negative association observed between bone mass and insulin resistance in boys [5]. Body composition was described by weight and %FM, and both of these were significantly associated with TBLH BMC in the same direction for boys and girls (Table 3), although sex differences in body composition increase during puberty [31]. Body composition may influence the association between HOMA-IR and bone mass as suggested by some authors [6,7], but this was not evident in our study. The association between HOMA-IR and TBLH BMC was only slightly affected by the addition of body composition to the models. It can, however, be speculated if sex-differences in body fat distribution may play a role [11].

Further studies are though needed to clarify whether some of these sex-differences can be explained by differences in visceral-fat distribution or differences in hormones.

Physical activity was measured objectively for at least 3 days at follow-ups with accelerometers and assumed to be representative of the physical activity level of the participants. MVPA or VPA levels did not influence the association between HOMA-IR and TBLH BMC in boys. This is in line with the result from Lawlor et al. [32] who in a cross-sectional study showed that MVPA did not alter associations between fasting insulin and bone mass in adolescents aged 15–16 years. Insulin resistance generally increases during puberty [33], and children may also tend to be less active in later puberty, as found by Janz et al. [34] and our study (Table 1). Children and adolescents may be able to improve their insulin resistance by increasing the amount of time engaging in VPA [14,35] and, based on the observed associations, thereby potentially reduce some of the negative effects on bone mass. On the other hand, increasing the time spent engaging in VPA may also increase bone mass independently of HOMA-IR level. A greater amount of VPA may thus be able to counteract some of the observed negative association from HOMA-IR on TBLH BMC in adolescents, although no direct interaction was found. Further research is needed to clarify this.

No interaction between OC and HOMA-IR with TBLH BMC was found in this study, and OC did not influence the negative association between HOMA-IR and TBLH BMC in boys. OC was negatively associated with TBLH BMC in line with Erceg et al. [36], whereas other studies have reported diverse results from no association between OC

Table 2
Associations between TBLH BMC and HOMA-IR.

TBLH BMC	Model 1	Model 1b	Model 2	Model 3
	β	β	β	β
Baseline TBLH BMC (g)	−0.003	0.004	0.05#	0.0005
Bone area (cm^2)	1.6†	1.6†	1.3†	1.5†
Height (cm)	−9.4†	−9.6†	−11.5†	−9.6†
$Maturity_{boy}$ (years)	47.2†	51.3†	36.3†	52.3†
Girl	−61.9†	−97.6†	−32.0	−93.2†
Girl-by-maturity	−21.7†	−25.3†	−11.1‡	−25.5†
$HOMA\text{-}IR_{both\ sexes}$	−21.8†			
$HOMA\text{-}IR_{boy}$		−35.2†	−28.1†	−32.5†
Girl-by-HOMA-IR		27.2#	25.9‡	29.3‡
Weight (kg)			10.4†	
%FM			−7.7†	
VPA				8.2†

Sex interactions calculated with boys as reference.
Number of participants (number of follow-up scans): model 1 + 2: n = 562 (694 DXA), model 3: n = 529 (642 DXA).
Model 1 = adjusted for sex, maturity, size (height and bone area) and baseline TBLH BMC level; Model 1b = adjusted for sex, maturity, size (height and bone area), baseline TBLH BMC level, and including an interaction term between HOMA-IR and sex; Model 2 = Model 1b + body composition (weight and % FM), Model 3 = Model 1b + VPA.
Abbreviations: TBLH BMC, total body less head bone mineral content; Bone area, total body less head bone area; Maturity, years from age at peak height velocity; Girl-by-maturity, interaction term between being a girl and maturity; HOMA-IR, homeostasis model assessment of insulin resistance; Girl-by-HOMA-IR, interaction term between being a girl and HOMA-IR; %FM, whole body percent fat mass; VPA, % time spent engaging in vigorous physical activity.
† Significant at $p < 0.001$.
‡ Significant at $p < 0.01$.
# Significant at $p < 0.05$.

Table 3
Associations between TBLH BMC and HOMA-IR stratified by sex.

	Model 1	Model 2	Model 3	Model 3b	Model 4	Model 5
	β	β	β	β	β	β
TBLH BMC **Boys**						
Baseline TBLH BMC (g)	−0.14†	−0.04	−0.16†	−0.16†	−0.07#	−0.13†
Bone area (cm^2)	1.7†	1.3†	1.7†	1.7†	1.3†	1.7†
Height (cm)	−10.3†	−10.9†	−10.2†	−10.2†	−10.5†	−9.1†
Maturity (years)	35.3†	20.4‡	35.6†	37.8†	21.7‡	31.5†
HOMA-IR	**−31.4**†	**−28.9**†	**−27.1**‡	**−29.1**‡	**−30.2**‡	**−29.3**†
Weight (kg)		12.4†			11.6†	
%FM		−8.3†			−7.3†	
VPA			10.2‡		5.8*	
MVPA				4.5‡		
OC (μg/l)						−0.32†
TBLH BMC **Girls**						
Baseline TBLH BMC (g)	0.18†	0.17†	0.18†	0.19†	0.16†	0.18†
Bone area (cm^2)	1.4†	1.3†	1.4†	1.4†	1.3†	1.4†
Height (cm)	−9.5†	−11.7†	−9.6†	−9.5†	−11.6†	−8.5†
Maturity (years)	44.0†	40.0†	45.3†	46.2†	40.3†	36.4†
HOMA-IR	−2.9	2.4	1.9	1.3	4.2	−1.5
Weight (kg)		7.4†			7.1†	
%FM		−6.0†			−5.4†	
VPA			8.4‡		5.3*	
MVPA				3.6#		
OC (μg/l)						−0.19#

Number of participants (number of follow-up scans): Boys: Model 1 + 2: n = 277 (346 DXA), model 3 + 4: n = 250 (308 DXA), model 5: n = 275 (340 DXA); Girls: Model 1 + 2: n = 285 (348 DXA), model 3 + 4: n = 279 (334 DXA), model 5: n = 282 (342 DXA).
Model 1 = Adjusted for maturity, size (height and bone area) and baseline TBLH BMC level; Model 2 = Model 1 + body composition (weight and %FM); Model 3 = Model 1 + VPA; Model 3b = Model 1 + MVPA; Model 4 = Model 1 + VPA + body composition; Model 5 = Model 1 + OC.
Abbreviations: TBLH BMC, total body less head bone mineral content; Bone area, total body less head bone area; Maturity, years from age at peak height velocity; HOMA-IR, homeostasis model assessment of insulin resistance; %FM, whole body percent fat mass; VPA, % time spent engaging in vigorous physical activity; MVPA, % time spent engaging in moderate to vigorous physical activity; OC, osteocalcin (μg/l).
† Significant at $p < 0.001$.
‡ Significant at $p < 0.01$.
# Significant at $p < 0.05$.
* p-Values for VPA in model 4: Boys p = 0.051, Girls p = 0.070.

with bone mass [37] to a positive association between OC and BMC [12,38]. The exact role of OC in glucose metabolism and bone remodelling in humans, and especially children, still remains to be elucidated [21].

4.1. Strengths and limitations

Strengths of this study include the long follow-up time spanning > 6 years as well as the inclusion of objectively measured physical activity and the hormone osteocalcin in analyses. The study had a large sample size with a total of 562 participants and 1256 DXA scans conducted on the same DXA device over the study period. All participants were apparently healthy, which increases our ability to generalise to the general youth population, although the low prevalence of obesity in the sample may require replication studies in populations with more obesity. Furthermore, although accelerometers may be superior to subjective measures in measuring physical activity [39], they are not able to measure water-based activities or activity of the upper body which may have altered the results.

Key limitations include a high drop-out rate before the last follow-up. Additionally, HOMA-IR was used as a surrogate measure of insulin resistance instead of a directly measure of insulin resistance. However, this method has proved to be a robust tool in epidemiological studies [26]. Total OC was used in analyses, as we were unable to measure ucOC that is thought to be the active part of osteocalcin in humans [18]. Other limitations are that calcium and vitamin D intake were not assessed, and that physical activity level was measured with accelerometers in 2010 and 2015 but not at baseline in 2008. The accelerometers do not provide an exact measure of physical activity at the individual-level but arguably provides the optimal balance between validity and feasibility available for epidemiological studies. We investigated the relationship between follow-up TBLH BMC and follow-up HOMA-IR, so our analyses do not provide a longitudinal interpretation of the exposure-outcome relation. However, the risk of reverse causality bias is reduced by controlling for baseline TBLH BMC.

5. Conclusions

A highly significant negative association was found between HOMA-IR and bone mass in boys, whereas no association was found in girls. The negative association in boys was not altered by body composition or physical activity, meaning that HOMA-IR might influence bone mass in a sex-dependent manner in children and adolescents independently of body composition or physical activity. Further studies are needed to better understand these sex differences.

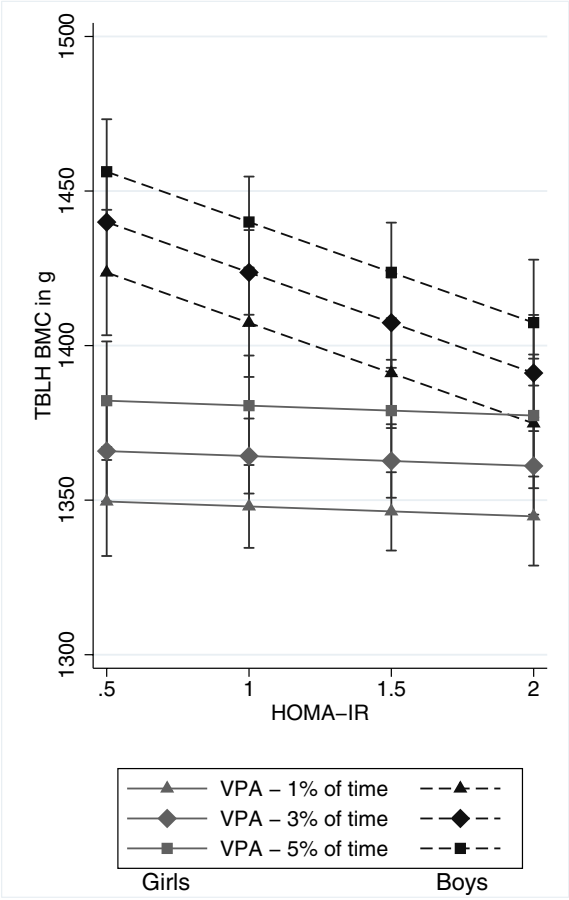

Fig. 2. Predicted values of TBLH BMC dependence on HOMA-IR and percent time spent engaging in vigorous physical activity (VPA).
Further adjusted for baseline TBLH BMC, bone area, height and sex.
Solid lines represent girls and dotted lines represent boys.
Abbreviations: TBLH BMC, total body less head bone mineral content; HOMA-IR, homeostasis model assessment of insulin resistance.

Declarations of interest

Maria Sode Rønne, Malene Heidemann, Louise Lylloff, Anders J. Schou, Jakob Tarp, Anna Bugge, Jens Ole Laursen, Niklas Rye Jørgensen, Steffen Husby, Niels Wedderkopp and Christian Mølgaard declare that they have no conflict of interest.

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