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The Bone Markers Sclerostin, Osteoprotegerin, and Bone-Specific Alkaline Phosphatase Are Related to Insulin Resistance in Children and Adolescents, Independent of Their Association with Growth and Obesity

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Keywords
Sclerostin · Osteoprotegerin · Bone-specific alkaline phosphatase · Obesity · Insulin resistance · Growth · Children

Abstract
Background/Aims: Sclerostin, osteoprotegerin, and bone-specific alkaline phosphatase (B-ALP), which are primarily related to bone metabolism, have been linked with insulin resistance in adults. We aimed to evaluate the association of these markers with growth, obesity, and parameters of insulin resistance in lean and obese children and adolescents. ***Methods:*** We measured sclerostin, osteoprotegerin, and B-ALP in fasting and oral glucose tolerance test (oGTT) serum samples from 1,325 children and adolescents, and during 24-h profiles and after exercise and glucose exposure in young adults. ***Results:*** In addition to the positive relationship with height standard deviation scores (SDS), sclerostin ($r = 0.035$, $p < 0.001$) and B-ALP ($r = 0.06$, $p = 0.028$) increased, whereas osteoprotegerin ($r = -0.098$, $p < 0.001$) decreased with BMI SDS. Furthermore, B-ALP correlated with fasting- and oGTT-derived markers of glucose and insulin metabolism suggestive of insulin resistance. To evaluate potential confounding diurnal variation of bone markers, we performed 24-h profiles. B-ALP and osteoprotegerin had lower night-time levels. Exercise acutely and transiently increased B-ALP and osteoprotegerin levels, but glucose ingestion had no effect. ***Conclusions:*** Besides their association with growth, sclerostin and osteoprotegerin levels are altered in childhood obesity. Particularly B-ALP was related to insulin resistance indices. Our findings accent the link between bone, growth, and insulin resistance.

Introduction

The worldwide increasing prevalence of obesity in all age groups [1] has led to the increased occurrence of metabolic consequences, particularly type 2 diabetes. The etiology and pathogenesis of insulin resistance are not yet fully understood, and many factors may play a role here.

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Insulin resistance is accompanied by alterations in many hormones, markers, receptors, and signaling pathways. Well-known is the association of obesity and various adipokines, i.e., proteins produced by adipose tissue [2], which contribute to insulin resistance. Nevertheless, several non-adipose-derived circulating molecules are suspected to contribute to obesity and insulin resistance [3]. Considering that childhood obesity is often accompanied by accelerated growth and that bone and adipose tissue are embryologically derived from common precursor cells, bone-derived factors came into focus. Recently, it has been shown that cytokines produced by bone (e.g., osteopontin and osteocalcin) might participate in energy metabolism, insulin resistance, and growth [4]. Some newer bone markers such as sclerostin and osteoprotegerin have been associated with insulin resistance and prediabetes in adults [4].

Sclerostin is a circulating cytokine produced by the liver, bones, kidneys, and cartilage, and the main target tissue for sclerostin is the bone where it inhibits osteoblast function [5]. Sclerostin serum concentrations are not influenced by age in children and adolescents [6]. The main effects of sclerostin are mediated by the inhibition of the Wnt (Wingless-type mouse mammary tumor virus integration side) signaling pathway [7]. This pathway interacts with insulin secretion and activity, and it has been implicated in various nonskeletal diseases like type 2 diabetes, obesity, early coronary disease, and cancer [8]. Recently, a positive correlation of circulating sclerostin levels with homeostasis model assessment of insulin resistance (HOMA-IR) and a negative association with insulin-mediated total body glucose has been shown in adults with prediabetes, indicating a link to insulin resistance [4]. The role of sclerostin in the early onset of insulin resistance, particularly in children, is unknown so far.

Osteoprotegerin is a circulating glycoprotein (encoded by the *TNFRSF11B* gene) and belongs to the TNF receptor superfamily. It is produced by osteoblasts and functions as a negative regulator of bone resorption by decreasing osteoclast development [9]. In a study on 70 children and adolescents, osteoprotegerin did not correlate significantly with age, height, or BMI [10]. In several studies, serum osteoprotegerin has been found to increase with liver fat content [11], and osteoprotegerin levels are higher in adults with diabetes or prediabetes than in those with normal glucose tolerance [12]. In obese adolescents, there was a positive correlation of osteoprotegerin with several parameters of insulin resistance (i.e., fasting serum insulin and HOMA-IR) [13], which was, however, not confirmed by another study including children and adolescents [14]. Another study [15] found that osteoprotegerin levels increased parallel with the physiological increase of insulin resistance during puberty [16].

Finally, bone-specific alkaline phosphatase (B-ALP) is an enzyme involved in bone mineralization. It is produced by osteoblasts via alternative splicing of the *ALPL* gene product. B-ALP concentrations are significantly influenced by age in children and adolescents, with the highest concentrations found during adolescence [6, 17]. Higher serum concentrations of both the total and intestinal isoform of ALP have been linked with obesity [18], but there are far fewer data on the bone-specific isoform in adults [19] and no data about children.

As several osteokines linked with insulin resistance and bone metabolism are associated with linear growth in children [3], we hypothesized that sclerostin, osteoprotegerin, and B-ALP levels are not only associated with linear growth but potentially with the development of insulin resistance in obese children. We aimed to evaluate the association of sclerostin, osteoprotegerin, and B-ALP levels with growth and parameters of insulin resistance in children and adolescents.

Materials and Methods

Study Subjects

Cohort for the Associations of Bone Markers with Growth and Metabolic Parameters

The associations with growth and metabolic parameters were studied in a cohort that included 1,399 probands from the Leipzig LIFE Child cohort [20]. We excluded 19 probands that had type 1 diabetes mellitus or were being treated with metformin, growth hormones, systemic corticosteroids, or immunosuppressants, and 52 underweight individuals (BMI standard deviation score [SDS] <1.88). This left a total of 1,325 children and adolescents aged 0.9–18 years (109 aged <5 years, 508 aged 5–10 years, 573 aged 10–15 years, and 135 aged >15 years). Clinical features of the probands are shown in online supplementary Table S1. 1a (for all online suppl. material, see www.karger.com/doi/10.1159/000497113). In a subgroup of probands (n = 239), we had additional data on parameters of insulin resistance derived from the fasting levels and an oral glucose tolerance test (oGTT). In 11 lean individuals (BMI SDS −0.3 ± 0.7) aged 12.8 ± 3.1 years, we determined serum levels of bone markers by oGTT at 0, 30, 60, 90, and 120 min (online suppl. Fig. S2).

Cohort for the Associations of Bone Markers with Growth and Weight Loss

To study the impact of weight loss and changes in insulin resistance, we included 58 obese individuals (aged 12.7 ± 2.3 years, BMI SDS 2.3 ± 0.4) from the Leipzig Childhood obesity intervention cohort, who were enrolled at the Leipzig obesity outpatient department and participated in a lifestyle intervention program for 4–6 weeks that included dietary changes and physical activity training.

Cohort for the 24-Hour Serum Profiles and Impact of Exercise on Bone Markers in Young Adults

The 24-h serum profiles of the bone markers and levels after a bout of acute strenuous exercise [21] were assessed in 4 young adults with no reported chronic disease (2 lean individuals [BMI 20.5–21.2] aged 20 and 21 years and 2 obese individuals [BMI 35.5 and 35.7] aged 32 and 34 years.

Anthropometry, Body Composition, and Pubertal Development

Height, BMI, and other anthropometric values were standardized by referring to sex- and age-specific national reference data [22]. Body fat percentage was measured using the bioelectrical impedance analysis method on the Biacorpus RX 400 (Idiag AG, Fehraltorf, Switzerland).

Pubertal status was evaluated by trained physicians using the Tanner criteria, and was stratified into 3 categories: prepubertal (P1), pubertal (P2–P4), and postpubertal (adolescent) (P5). Blood pressure values were standardized by referring to sex, age, and height [23].

Biochemical Analyses

Sclerostin, osteoprotegerin, and B-ALP serum concentrations were measured from fasting serum samples by the human bone panel I multiplex electrochemiluminescence immunoassay (Mesoscale Discovery). Sclerostin measurements in the range of 0.004–100 ng/mL demonstrated interassay coefficient of variation (CV) values of 10.9% at 0.03 ng/mL and 2.3% at 5.38 ng/mL. Osteoprotegerin measurements in the range of 0.004–200 ng/mL demonstrated interassay CV values of 10.1% at 0.16 ng/mL and 6.1% at 5.81 ng/mL. B-ALP measurements in the range of 1.9–4,000 ng/mL demonstrated interassay CV values of 6.1% at 6.0 ng/mL and 6.8% at 74.1 ng/mL.

Insulin-like growth factor 1 (IGF1) and IGF-binding protein 3 (IGFBP3) measurements were performed with an automated chemiluminescence immunoassay method (IDS-iSYS, London, UK). The raw data of concentration were standardized to gender- and age-dependent SDS using the reference data of the manufacturer [24]. Interassay CVs were between 2.4 and 7.2% for IGF1 levels in the range of 31.2–870 ng/mL and between 6.0 and 8.6% for IGFBP3 levels in the range of 2.06–5.06 μg/mL.

Serum glucose concentrations were measured by the photometric method (Roche, Basel, Switzerland). Serum insulin concentrations were measured using a quantitative electrochemiluminescence method (Roche) or an automated chemiluminescence immunoassay (DiaSorin, Saluggia, Italy). For glucose and insulin measurements, both the fasting levels and oGTT with 75 g glucose were performed using a standardized WHO protocol following a 10-h overnight fast [25].

Glycosylated hemoglobin (HbA_{1c}) was evaluated from whole blood by the immunturbidimetric method with a Roche-Cobas analyzer (Roche). All the values were transformed into Diabetes Control and Complications Trial (DCCT percentage) and International Federation of Clinical Chemistry (IFCC) units [26]. Lipids were measured by standard laboratory protocols in the Institute of Laboratory Medicine, University of Leipzig, Germany.

For the assessment of insulin secretion and/or resistance, we selected fasting- and oGTT-derived indices commonly used for characterizing insulin secretion and insulin resistance (online suppl. Table S2): fasting insulin (INS_0), HOMA-IR, and the product of fasting triglycerides and glucose levels (TyG), 2-h insulin during an oGTT (INS_{120}), peak insulin level during an oGTT (INS_{max}), the ratio of the area under the curve for insulin and glucose levels during an oGTT (AUC_{INS}/AUC_{GLU}), and the Matsuda whole-body insulin sensitivity index (WBISI). Where appropriate, glucose and insulin concentrations were transformed from mmol/L and nmol/L to mg/dL and ng/mL using the coefficient 0.05551 for glucose and 6.945 for insulin, respectively.

Statistical Analyses

Values for the cohort description are given as mean ± SD. Non-normally distributed parameters were logarithmically transformed prior to further statistical analyses. Comparisons between group means were tested using the Student *t* test and ANOVA. Anthropometric, metabolic, and insulin resistance parameters, sclerostin, osteoprotegerin, and B-ALP levels were included in the Pearson partial correlation to control for sex, age, height SDS, BMI SDS, and pubertal development. Multiple regression analysis with stepwise forward model selection was performed using height SDS, fasting insulin, TyG, HOMA-IR, 2-h oGTT insulin, AUC_{INS}/AUC_{GLU}, and WBISI as dependent variables. As covariables, we included sex, age, BMI SDS, pubertal state, sclerostin, osteoprotegerin, B-ALP, IGF1 SDS, and IGFBP3 SDS for height SDS, and HbA_{1c} for the remaining analyses (all of the covariables were previously tested in the Pearson correlation and univariate regression analysis; $p < 0.2$). $p < 0.05$ was considered statistically significant. In the case of multiple testing, the Bonferroni correction was used. Statistical analyses were performed with STATISTICA 10 software (Dell, Round Rock, USA).

Results

Association of Sclerostin and Osteoprotegerin with Growth and Obesity

We first assessed the (expected) relationship of the bone markers with age and height SDS as parameters related to growth (Table 1). In univariate correlation analyses, sclerostin and B-ALP increased with height SDS (Fig. 1). These associations remained significant independent of sex, age, and puberty in the multiple linear regression models (online suppl. Table S3). For osteoprotegerin, we saw a borderline negative correlation with height SDS, which was lost after correcting for cofactors in multiple regression models (Fig. 1; online suppl. Table S3).

Sclerostin level was 29.3% higher in obese than in normal-weight children (online suppl. Table S1) and increased with BMI SDS, independent of the covariates of physical development (online suppl. Table S3), whereas osteoprotegerin was lower in obese children and decreased with BMI SDS (Fig. 1, online suppl. Table S3). B-ALP levels were similar in obese and normal-weight children, and there was no correlation with BMI SDS after adjusting for age and puberty (online suppl. Table S3).

Table 1. Partial correlation of sclerostin, osteoprotegerin, and B-ALP with selected parameters of body composition and growth mediators controlled for age, sex, height SDS, BMI SDS, and pubertal development

	Sclerostin			Osteoprotegerin			B-ALP		
	n	*r*	*p*	*n*	*r*	*p*	*n*	*r*	*p*
Waist SDS	1,290	0.052	0.071	1,292	–0.030	0.303	1,292	–0.036	0.274
Hip SDS	1,281	0.029	0.319	1,283	–0.050	0.081	1,283	–0.080	**0.006**
Waist-to-hip ratio SDS	635	0.148	*<0.001*	635	0.030	0.471	635	0.024	0.562
Body fat, %	295	0.099	0.114	295	–0.204	*0.001*	295	–0.509	*<0.001*
IGF1	1,303	0.008	0.784	1,305	–0.064	**0.026**	1,304	0.222	*<0.001*
IGF1 SDS	1,303	0.012	0.663	1,305	–0.041	0.154	1,304	0.149	*<0.001*
IGFBP3	1,297	0.066	**0.022**	1,299	0.105	*<0.001*	1,298	0.198	*<0.001*
IGFBP3 SDS	1,297	0.083	*0.004*	1,293	0.113	*<0.001*	1,298	0.171	*<0.001*
Sclerostin	n.a.	n.a.	n.a.	1,323	0.354	*<0.001*	1,322	0.187	*<0.001*
Osteoprotegerin	1,323	0.354	*<0.001*	n.a.	n.a.	n.a.	1,324	0.253	*<0.001*
B-ALP	1,322	0.187	*<0.001*	1,324	0.253	*<0.001*	n.a.	n.a.	n.a.

The significant correlations ($p < 0.05$) are marked in bold, and significant correlations after the Bonferroni correction ($p < 0.005$) are marked in italics. n.a., not available; SDS, standard deviation score; IGF1, insulin-like growth factor 1; IGFBP3, insulin-like growth factor-binding protein 3; B-ALP, bone-specific alkaline phosphatase.

As obesity is often associated with accelerated growth, we performed multiple regression analyses to statistically dissect the interrelationships. BMI SDS was a strong predictor of height SDS and, independent of this, all 3 bone markers were related to height SDS. Sclerostin ($\beta = 0.07$, $p = 0.012$) and B-ALP ($\beta = 0.15$, $p < 0.001$) were significantly positively associated with height SDS and osteoprotegerin was negatively associated ($\beta = -0.08$, $p = 0.003$) (online suppl. Table S4).

Overall, sclerostin increased and osteoprotegerin decreased with obesity, whereas B-ALP was primarily associated with growth.

Diurnal Variation and Effect of Glucose and Exercise Provocation on Bone Markers in Young Adults

Before assessing potential relationships of bone marker serum levels with the metabolic state in lean and obese children, we assessed the natural diurnal variation and the acute effects of glucose ingestion and physical exercise on sclerostin, osteoprotegerin, and B-ALP levels. During the day, there was no characteristic pattern, but serum levels of B-ALP and osteoprotegerin were significantly lower during the night hours (online suppl. Fig. S1). This was confirmed by a direct comparison of mean daytime versus night-time levels, as higher levels during the day may be the result of exercise or meals. Acute exercise led to an immediate increase in B-ALP and osteoprotegerin levels (online suppl. Fig. S2). However, glucose ingestion did not affect serum levels of bone markers during oGTT (online suppl. Fig. S2).

Association of Bone Markers with Metabolic Parameters and Indices of Insulin Resistance

We analyzed the correlation of serum levels of the 3 bone markers by applying partial correlation analyses adjusted for age, sex, BMI SDS, and pubertal development (Table 2). B-ALP correlated positively with markers of impaired glucose and insulin metabolism and triglyceride levels and decreased with the insulin sensitivity index. We observed some significant correlations for sclerostin and osteoprotegerin that did not withstand the Bonferroni correction.

When we restricted the analyses to the obese subpopulation, as it is more prone to alterations in glucose metabolism and insulin resistance, there were no significant correlations with sclerostin, but osteoprotegerin correlated with fasting serum insulin ($r = 0.237$, $p = 0.018$) and WBISI ($r = -0.214$, $p = 0.044$); and B-ALP with TyG index ($r = 0.201$, $p = 0.034$), fasting serum insulin ($r = 0.239$, $p = 0.011$), 2-h oGTT insulin ($r = 0.198$, $p = 0.044$), WBISI ($r = -0.298$, $p = 0.005$), and AUC_{INS}/AUC_{GLU} ($r = 0.312$, $p = 0.003$). Nevertheless, only the association of B-ALP with fasting glucose, TyG, 2-h oGTT insulin, WBISI, and AUC_{INS}/AUC_{GLU} in all cases, and B-ALP with AUC_{INS}/AUC_{GLU} in obese individuals, survived Bonferroni correction.

We performed multiple regression analyses to assess whether the bone markers (independently) contributed to variations in metabolic parameters, which confirmed the association of B-ALP with fasting ($\beta = 0.13$, $p < 0.001$)

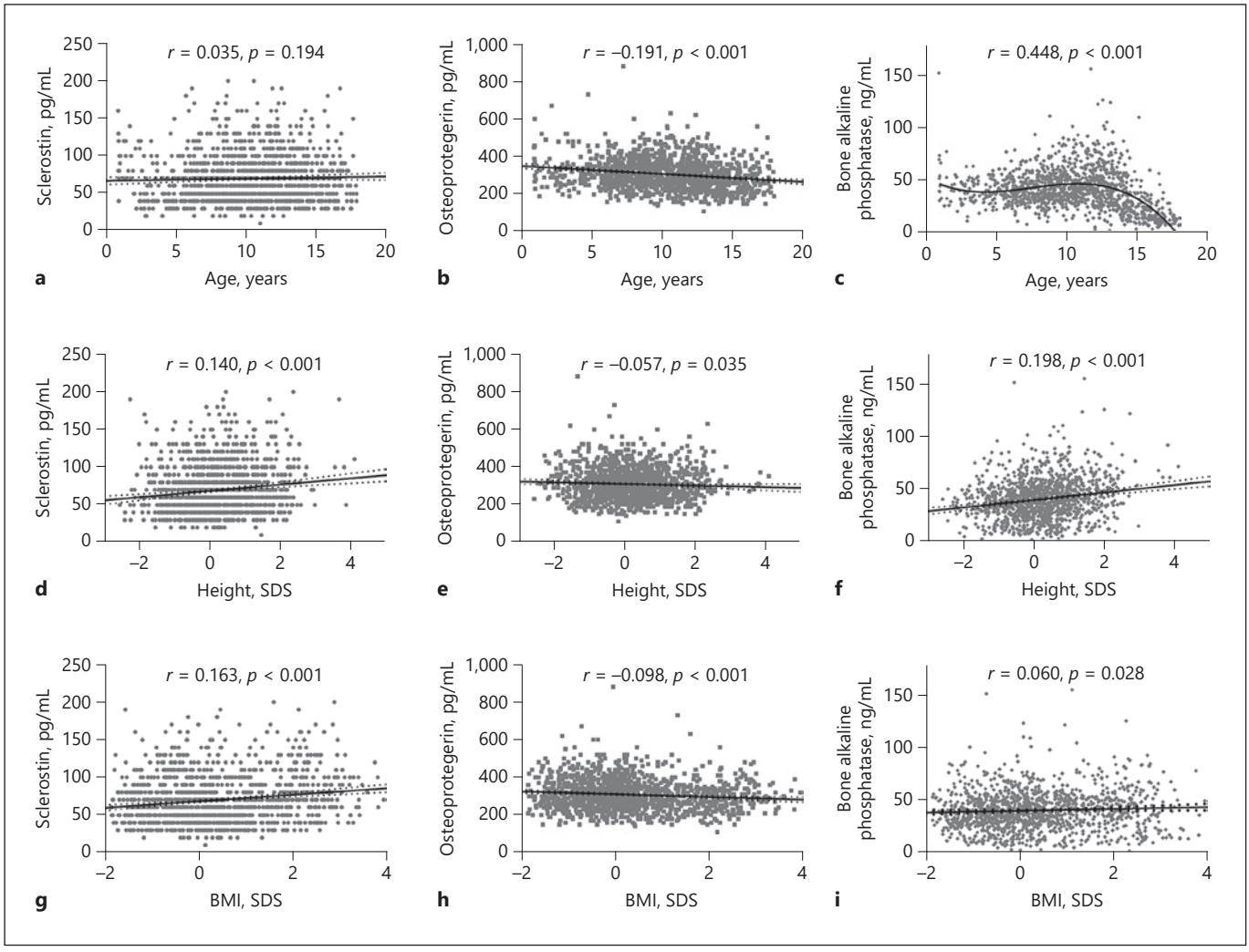

Fig. 1. Association of sclerostin (**a**), osteoprotegerin (**b**), and bone-specific alkaline phosphatase (B-ALP, **c**) with age. Association of sclerostin (**d**), osteoprotegerin (**e**), and B-ALP (**f**) with height SDS. Association of sclerostin (**g**), osteoprotegerin (**h**), and B-ALP (**i**) with BMI SDS. **a**, **b**, **d–i** *r*, Pearson correlation coefficient. **c** *r* coefficient and curve estimation were calculated by polynomial cubic regression because of nonlinear association.

and stimulated (β = 0.17, $p = 0.027$) glucose and insulin parameters, including fasting insulin (β = 0.15, $p < 0.001$), HOMA-IR (β = 0.10, $p = 0.007$), AUC_{INS}/AUC_{GLU} (β = 0.15, $p = 0.012$), and WBISI (β = –0.2, $p < 0.001$), independent of anthropometric and developmental parameters (online suppl. Table S5). Sclerostin significantly contributed to glucose-stimulated insulin secretion (β = 0.13, $p = 0.018$) and AUC_{INS}/AUC_{GLU} (β = 0.14, p = 0.004) (online suppl. Table S5).

Hence, the classical bone marker B-ALP, in particular, was BMI-independently associated with metabolic parameters of insulin resistance, and sclerostin was primarily associated with glucose-stimulated insulin secretion.

Impact of Weight Loss Intervention on Bone Markers

A 6-week classical in-house weight loss intervention in 58 obese children resulted in a significant weight loss of 4.8 ± 0.33 kg, relating to –0.26 ± 0.01 BMI SDS, which did, however, not affect the serum levels of the bone markers B-ALP, sclerostin, and osteoprotegerin. Furthermore, there were no significant correlations between changes in BMI SDS or selected insulin resistance indices with changes in sclerostin, osteoprotegerin, or B-ALP levels.

Table 2. Partial correlation of sclerostin and osteoprotegerin with selected parameters of insulin resistance and metabolic health in children and adolescents who were of normal weight, overweight, or obese

	Sclerostin			Osteoprotegerin			B-ALP		
	n	*r*	*p*	*n*	*r*	*p*	*n*	*r*	*p*
HbA_{1c}	1,248	0.099	**0.015**	1,250	0.060	0.141	1,249	0.087	*0.003*
Systolic blood pressure SDS	1,305	0.030	0.467	1,307	0.050	0.215	1,307	0.003	0.929
Diastolic blood pressure SDS	1,305	0.064	0.113	1,307	0.025	0.534	1,307	–0.055	0.053
Triglycerides	1,254	0.111	**0.006**	1,256	0.052	0.199	1,255	0.084	**0.004**
Total cholesterol	1,255	0.036	0.371	1,257	0.049	0.23	1,256	0.031	0.288
HDL cholesterol	1,257	–0.044	0.277	1,259	0.012	0.762	1,258	–0.021	0.474
Fasting serum glucose	1,248	0.002	0.953	1,250	0.048	0.237	1,249	0.116	*<0.001*
2-h oGTT glucose	246	0.035	0.674	246	0.070	0.394	246	0.131	**0.047**
TyG	1,239	0.104	**0.010**	1,241	0.054	0.185	1,240	0.116	*<0.001*
Fasting serum insulin	490	–0.064	0.367	490	0.018	0.801	490	0.190	*<0.001*
2-h oGTT insulin	239	0.139	**0.050**	239	0.183	**0.009**	239	0.153	**0.022**
INS_{max}	233	0.085	0.221	233	0.109	0.111	233	0.190	**0.005**
HOMA-IR	474	–0.064	0.368	474	0.032	0.657	474	0.120	**0.012**
WBISI	219	–0.065	0.361	219	–0.114	0.106	219	–0.284	*<0.001*
AUC_{INS}/AUC_{GLU}	218	0.143	**0.042**	218	0.142	**0.045**	218	0.208	*0.003*

Controlled for sex, age, height SDS, BMI SDS, and pubertal status, and insulin for the insulin method. Significant correlations ($p < 0.05$) are marked in bold, and significant correlations after the Bonferroni correction ($p < 0.003$) are marked in italics. Hb, hemoglobin; HDL, high-density lipoprotein; SDS, standard deviation score; 2-h oGTT glucose, 120-min value of glucose during a 75-g oral glucose tolerance test; TyG, the product of triglycerides and fasting glucose; 2-h oGTT insulin, 120-min value of insulin during a 75-g oral glucose tolerance test; INS_{max}, peak insulin level during an oGTT; HOMA-IR, homeostasis model assessment of insulin resistance; AUC_{INS}/AUC_{GLU}, ratio of area under the curve for insulin and glucose levels during a 75-g oral glucose tolerance test; WBISI, whole-body insulin sensitivity index.

Discussion

This study shows that the serum levels of the bone markers sclerostin, osteoprotegerin, and B-ALP in children and adolescents are not only related to height SDS, but also to BMI SDS. Independent of these associations with anthropometric parameters, the classical bone marker B-ALP, in particular, was related to levels of fasting and stimulated glucose and insulin and selected insulin resistance indices. Despite an association with BMI, there was no major independent relationship of sclerostin and osteoprotegerin with glucose and insulin metabolism.

This is, so far, the largest study focusing on the association of sclerostin and osteoprotegerin levels with anthropometric parameters. In contrast to a previous study [6], we found a positive correlation of sclerostin with BMI SDS and height SDS. This could be explained by the greater number of individuals included in our study. The association of osteoprotegerin with BMI SDS was not evidently similar to previous studies [27], and the lack of association of osteoprotegerin levels with BMI SDS in smaller-scale studies may fit the overall picture that osteoprotegerin is not majorly related to obesity [10].

In general, the association of cytokines (e.g., osteocalcin) with bone metabolism and growth is known and is to be expected [28]. Here, we added data on sclerostin and osteoprotegerin as independent predictors of height SDS. This link could be partially caused by the association of sclerostin and osteoprotegerin with insulin metabolism (see below), and we also speculated about the influence of skeletal size on sclerostin levels. Moreover, our results support previously published results on the association of B-ALP and growth [29].

Sclerostin and osteoprotegerin have been associated with early forms of developing diabetes and insulin resistance in adults [4, 30]. Hence, it is of interest to study this relationship in (obese) children and adolescents, who frequently develop insulin resistance but are less biased by confounding factors such as medications.

We found an association of sclerostin levels with several insulin resistance indices. Our results support the hypothesis of Daniele et al. [4] that sclerostin could be associated with insulin activity and clearance, as we primarily saw a relationship with glucose-stimulated insulin secretion. On the other hand, all significant correlations between sclerostin and insulin resistance indices (i.e.,

TyG, 2-h oGTT insulin, and AUC_{INS}/AUC_{GLU}) were mild ($r < 0.2$), in agreement with Ugur-Altun et al. [31], and much weaker than the findings of Daniele et al. [4]. Moreover, we did not find a significant association between sclerostin and fasting levels of glucose, insulin, and HOMA-IR, as described in both previous publications [4, 30]. This may indicate that glucose-stimulated insulin secretion is impaired before fasting levels are affected. The discrepancy could be caused by several issues, i.e., both previous studies involved adult participants only, with prediabetes [4] or type 2 diabetes [30], which supposedly show more severe alterations in glucose metabolism. Overall, their results and our linear regression results, proving that sclerostin is an independent predictor of insulin secretion during oGTT, support the link between sclerostin level and insulin resistance.

Regarding the association of osteoprotegerin with insulin resistance, we found only mild associations with oGTT-derived indices (particularly 2-h oGTT insulin in the linear regression analysis). Two previously published studies, involving a smaller number of adolescents ($n = 98$) [13] and adults ($n = 74$) [31], respectively, found associations of osteoprotegerin levels with fasting insulin and HOMA-IR, which we did not observe. Our results are consistent with those of Erol et al. [14] in 174 children and adolescents; they failed to find an association of osteoprotegerin with HOMA-IR. Though there is a relationship between osteoprotegerin and liver fat in adults [11] which constitutes a risk factor for insulin resistance, the relevance of this bone marker for glucose metabolism, particularly in children and adolescents, remains unclear and is probably minimal.

Surprisingly, we found that the strongest associations with the majority of the insulin and glucose metabolism parameters were with the most "classical" bone marker, B-ALP. In previous studies, ALP serum levels have been associated with obesity [18] and a fatty liver [32], but the studies concentrated on total levels rather than differentiating between distinct tissue-specific isoforms, or else on intestinal ALP. Together with the findings of Cheung et al. [19], who found a positive significant association of B-ALP levels with insulin and HOMA-IR, our data may support a link between B-ALP and glucose and insulin metabolism independent of growth and the extent of obesity.

Many growth-related and metabolic factors are subject to variation due to exogenous stimuli. We studied these factors precisely by assessing the effect of glucose stimulation during an oGTT but did not find any dynamics.

One of the limitations of our study was the small number of the participants in 2 of the subgroups, and the fact that 1 subgroup analysis was conducted on young adults.

Conclusion

Besides their association with growth, sclerostin and osteoprotegerin levels are slightly altered in childhood obesity. Independent of BMI SDS, the classical bone marker B-ALP, in particular, was related to insulin resistance indices. Our findings accent the link between bone, growth, and insulin resistance. However, further prospective studies will be needed to confirm the associations of sclerostin, osteoprotegerin, and B-ALP with parameters of growth and insulin resistance in children and adolescents.

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Statement of Ethics

Written consent was obtained from parents and in children and adolescents >12 years also their assent. All studies have been approved by the local ethics committee.

Disclosure Statement

The authors have nothing to disclose.

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Author Contributions

Study design: AK, JK, and JS. Data collection: MV. Data analysis: JK and JT. Data interpretation: AK, JK, JS, and AF. Drafting of the manuscript: TC and DD; revision of content: RF and AF; and approval of the final version: TC, DD, RF, and AF. RF takes responsibility for the integrity of the data analysis.

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