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Clinical Research Article

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Impact of Lean Body Mass and Insulin Sensitivity on the IGF-1–Bone Mass Axis in Adolescence: the EPICOM Study

Rikke Beck Jensen,[1] Birgitte Bytoft,[2] Zuzana Lohse,[3] Sine Knorr Johnsen,[4] Morten Frost Nielsen,[5] Peter Sandor Oturai,[6] Kurt Højlund,[3,7] Peter Damm,[2,8] Tine D. Clausen,[8,9] and Dorte M. Jensen[3,7,10]

[1]Department of Growth and Reproduction, Rigshospitalet, University of Copenhagen, Copenhagen DK-2100, Denmark; [2]Center for Pregnant Women with Diabetes, Department of Obstetrics, Rigshospitalet, University of Copenhagen, Copenhagen DK-2100, Denmark; [3]Steno Diabetes Center Odense, Odense University Hospital, Odense DK-5000, Denmark; [4]Department of Endocrinology and Internal Medicine, Aarhus University Hospital, Aarhus DK-8200, Denmark; [5]Department of Endocrinology & KMEB Molecular Endocrinology Unit, Odense University Hospital, Odense DK-5000, Denmark; [6]Department of Clinical Physiology, Nuclear Medicine and PET, Rigshospitalet, University of Copenhagen, Copenhagen DK-2100, Denmark; [7]Department of Clinical Research, Faculty of Health Sciences, University of Southern Denmark, Odense DK-5000, Denmark; [8]Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen DK-2100, Denmark; [9]Department of Gynecology and Obstetrics, Nordsjællands Hospital Hillerød, Denmark; and [10]Department of Gynecology and Obstetrics, Odense University Hospital, Odense DK-5000, Denmark

ORCiD numbers: 0000-0002-4522-672X (R. B. Jensen); 0000-0002-5615-4354 (B. Bytoft); 0000-0002-9828-9185 (P. S. Oturai); 0000-0002-0891-4224 (K. Højlund); 0000-0002-2067-5246 (P. Damm); 0000-0001-5585-2153 (T. D. Clausen); 0000-0002-3298-9824 (D. M. Jensen).

Abbreviations: BIGTT-AIR, oral glucose tolerance test–derived index of acute insulin response; BIGTT-IS, oral glucose tolerance test–derived index of insulin sensitivity; BMC, bone mineral content; BMD, bone mineral density; BMI, body mass index; DEXA, dual-energy x-ray absorptiometry; EPICOM, Epigenetic, Genetic and Environmental Effects on Growth, Metabolism and Cognitive Functions in Offspring of Women with Type 1 Diabetes; GH, growth hormone; HOMA-IR, homeostatic model assessment of insulin resistance; IGF-1, insulin-like growth factor-1; IGFBP-3, insulin-like growth factor-binding protein 3; OGTT, oral glucose tolerance test; pQCT, peripheral quantitative computed tomography; T1D, type 1 diabetes.

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Abstract

Context: Insulin-like growth factor-1 (IGF-1) is involved in the growth of muscle and bone mass and contributes to glucose homeostasis. The offspring of mothers with diabetes during pregnancy have an increased risk of insulin resistance (IR).

Objective: We hypothesized that bone mass was decreased in the offspring of mothers with type 1 diabetes (T1D), and that the IGF-1–bone mass relationship would be negatively influenced by IR.

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Design: Data from the Epigenetic, Genetic and Environmental Effects on Growth, Metabolism and Cognitive Functions in Offspring of Women with Type 1 Diabetes (EPICOM) study performed from 2012 to 2013 were included.
Setting: This work is a follow-up study of a nationwide register study.
Patients: A total of 278 adolescent index offspring whose mothers had T1D and 303 matched controls were studied.
Main Outcome Measure: Bone mineral content (BMC) determined by a dual-energy x-ray absorptiometry scan and the interaction with IGF-1 and insulin sensitivity were measured.
Results: There was no difference in BMC, bone mineral density, height (SD score [SDS]), or BMC/height between index and control offspring. IGF-1 (SDS) did not differ between the groups but insulin-like growth factor-binding protein 3 (SDS) was higher in index boys compared to controls (B = .31 [95% CI, 0.06-0.57], $P = .02$). The statistical path analysis showed that IGF-1 predicted BMC/height (B = .24 [95% CI, 0.02-0.45], $P = .03$), but lean mass was a mediator of this. IGF-1 and the homeostatic model assessment of IR were positively associated (B = .75 [95% CI, 0.37-1.12], $P < .001$). There was no moderating effect of the interaction between IR and IGF-1 on lean mass in the entire cohort (B = .005 [95% CI, –0.03 to 0.04], $P = .81$) or when analyzing index cases and controls separately.
Conclusion: We found that lean mass was an intermediary factor in the IGF-1–bone mass relationship in a large cohort of adolescents, and this relationship was not moderated by IR.

Freeform/Key Words: insulin-like growth factor-1 (IGF-1), insulin sensitivity, bone mineral content

Fluctuation of insulin sensitivity occurs during pubertal development reflecting the interplay between insulin metabolism, the growth hormone/insulin-like growth factor-1 (GH/IGF-1) axis, and sex steroids. Insulin sensitivity decreases before physical signs of puberty and before increases in sex steroid levels are detectable (1). The decrease in insulin sensitivity may partly be explained by increasing adiposity before puberty but also by the physiological activation of the GH/IGF-1 axis, which contributes to a relative insulin resistance (1). IGF-1 levels increase through childhood with a steep incline during puberty until Tanner stage 4 is reached and decreases in Tanner stage 5 at the end of puberty (2). Changes in insulin sensitivity and IGF-1 concentrations during puberty follow the same pattern with a peak in midpuberty (2, 3).

IGF-1 is an important hormone for childhood growth involved in development, regulation, and cell proliferation of skeletal muscle and bone mass (4, 5). There is substantial evidence that IGF-1 plays an important role in osteoblast and osteoclast cell proliferation (6-8). However, it has also been suggested that IGF-1 promotes bone growth indirectly by the effect on skeletal muscle via the increased mechanical load to which the bone adapts its structure and mass. A study of transgenic mice that overexpressed IGF-1 in muscle found that the increased muscle mass was associated with increased cortical bone (9). Human studies of a longitudinal cohort of 258 girls followed through puberty found that IGF-1 was indirectly associated with bone mass accrual measured by peripheral quantitative computed tomography (pQCT) through stimulating muscle growth (10), and similarly Kindler et al concluded that lean mass was an intermediary factor in the IGF-1 bone relationship in 9- to 11-year-old girls (11).

Increasing evidence suggests that IGF-1 plays an important role in glucose metabolism (12). IGF-1 and insulin receptors share some homology and downstream signaling pathways, and insulin resistance may therefore have adverse effects on IGF-1–dependent processes. Studies have shown that obesity and increased insulin resistance during puberty may have a negative impact on bone mass and density in children (13-17). Insulin resistance has been proposed to be followed by "IGF-1 resistance" (18) and thereby it could be hypothesized that bone development is compromised both directly by the decreased proliferative effect of IGF-1 on osteoblasts (6) and also indirectly via suboptimal IGF-1–dependent muscle development in insulin-resistant children. One former study evaluated bone mass in the offspring of mothers with type 1 diabetes (T1D), who had a less favorable metabolic profile than controls, but they found no difference in areal bone mineral density (BMD) or volumetric BMD between offspring and controls (19).

The aim of the present study was to explore the association between IGF-1 and bone mineral content for height (BMC/height) including the mediating effect of

lean mass and the moderating effect of insulin resistance in the Epigenetic, Genetic and Environmental Effects on Growth, Metabolism and Cognitive Functions in Offspring of Women with Type 1 Diabetes (EPICOM) cohort. In the EPICOM cohort, we studied 278 index offspring age 13.0 to 20.4 years whose mothers had T1D during pregnancy and an age-matched control group of 303 adolescents (20, 21). We have previously shown that the index cases had a less favorable metabolic profile and higher frequency of prediabetes than the control group. We therefore hypothesized that the index cases would have decreased bone mass due to lack of the anabolic effect of IGF-1 and insulin on osteoblast proliferation and that the IGF-1–bone mass relationship would be negatively influenced by insulin resistance.

Materials and Methods

A nationwide registry, with data on all pregnancies in women with T1D in Denmark from 1993 to 1999, was used to invite the offspring of mothers with T1D to participate in a follow-up study during 2012 to 2013. For the present study only singletons and only the first child per mother were included and 746 children of women with T1D (index children) from the original cohort were eligible for the follow-up examination and invited to participate in the study (reported in detail previously [20]). A total of 278 index offspring and 303 control individuals from the background population were included in the study. Maternal pregestational or gestational diabetes was an exclusion criterium in the group of control individuals. The protocol was in accordance with the Declaration of Helsinki and approved by the local ethics committee (M-20110239). The study was registered at Clinicaltrials.gov (ID: NCT01559181).

Clinical examination

The participants were examined in 3 university hospital settings in Denmark (Copenhagen, Odense, and Aarhus) from April 2012 until October 2013, and the participants had a mean age of 16.7 years (range, 13.0-20.4 years). Participants were studied after an overnight fast. Anthropometric measurements (height, weight, and waist circumference) were performed as previously described (20) and a standard 2-hour oral glucose tolerance test (OGTT) was performed after collection of fasting blood samples. Height, weight, and body mass index (BMI) SD scores (SDS) were calculated using normal Danish reference material (22), and pubertal development was evaluated by inspection and palpation according to Marshall and Tanner (23, 24).

Total body fat percentage, lean mass, BMC, and BMD were determined using dual-energy x-ray absorptiometry (DEXA). The DEXA scans were performed using a GE Healthcare Lunar Prodigy whole-body scanner (model DF+350646; GE Medical Systems) in Copenhagen; and a Hologic whole-body scanner model Discovery A (Odense) or Discovery W (Aarhus), as previously described (21). Bone mass increases during puberty mainly because of increased statural growth and thereby increase in bone size (25). We therefore used the size-adjusted bone mass: BMC divided by height (g/cm).

Biochemical analyses

Plasma glucose was measured using a hexokinase-glucose-6-phosphate dehydrogenase assay (Abbott Diagnostics). Serum insulin was measured by the enzyme-linked immunosorbent assay method using dual-monoclonal antibodies (ALPCO Diagnostics). Serum IGF-1 and insulin-like growth factor-binding protein 3 (IGFBP-3) concentrations were determined by chemiluminescence technology (Immunodiagnostics Systems [IDS]-iSYS IGF-I and IDS-iSYS IGFBP-3 assays, IDS Ltd) on the IDS-iSYS Multi-Discipline automated analyzer (IDS-iSYS). The level of detection for serum IGF-1 and IGFBP-3 were 10 ng/mL and 80 ng/mL, respectively. None of the measurements of IGF-1 and IGFBP-3 were below the level of detection in this cohort. IGF-1 (SDS) and IGFBP-3 (SDS) were calculated using a normal reference population (unpublished data). Insulin sensitivity was evaluated by the OGTT-derived model for assessment of insulin sensitivity index (BIGTT-SI0-30-120) (26) and the homeostatic model assessment of insulin resistance (HOMA-IR) (27). To assess β-cell function, we calculated the OGTT-derived index of acute insulin response (BIGTT-AIR-0-30-120) (26).

Statistical analyses

Histograms of all variables were evaluated for outliers and non-normal distribution. Nonnormal distributions were corrected by log (HOMA-IR, fat mass, lean mass) transformations. A linear model was fitted for each of the outcomes with index/control status as an independent variable reporting the differences between the groups as estimates with 95% CI and *P* values. Data were adjusted for sex, age (excluding SDS indices), and pubertal stage. Log-transformed data are presented as differences between the groups given as a percentage.

A path analysis (model 4 mediation as described by Preacher et al [28]) was performed to determine whether the significant association between IGF-1 and BMC/height

was mediated through lean mass. Furthermore, we tested the moderating effect of HOMA-IR on the lean mass–mediated relationship between IGF-1 and BMC/height. BMC/height was regressed on lean mass, IGF-1, HOMA-IR, and IGF-1×HOMA-IR. Lean mass was regressed on IGF-I, HOMA-IR, and IGF-I×HOMA-IR. Pubertal staging was a covariate in all analyses. All *P* values less than .05 were considered statistically significant, and we used SPSS, version 25 (IBM Corp). Path analyses were performed using the SPSS PROCESS program (28).

Results

The entire cohort consisted of 581 participants (346 females): 278 index offspring and 303 control offspring. There was no difference in pubertal staging comparing the index cases to the controls (Pearson chi-square *P* = .49), and 90% of the participants were Tanner 4 or 5. BMC/height, lean mass, IGF-1, and HOMA-IR fluctuated throughout puberty (Fig. 1). BMC/height and lean mass increased from Tanner stage 2 to 5 (Fig. 1A), and the boys had higher BMC/height and lean mass than girls, especially in Tanner stages 4 and 5 (Fig. 1B). There was no difference for both sexes in height (SDS), BMC, BMC/height, or BMD between the index and control offspring adjusted for Tanner stage (Table 1). As previously shown, we found a higher weight (SDS), BMI (SDS), fat percentage, and HOMA-IR in the index group compared to controls (see Table 1), but this reached significance only among the girls. Insulin resistance determined by HOMA-IR was stable throughout puberty both in boys and girls, with a slightly higher HOMA-IR in girls than in boys (Fig. 1D). Insulin sensitivity determined by BIGTT-SI and insulin secretion determined by BIGTT-AIR were also stable throughout puberty for both sexes (data not shown), and BIGTT-SI was lower in the index group compared to controls (see Table 1). BIGTT-AIR was higher among the index cases, but this was significant only among the girls (see Table 1). Serum IGF-1 concentrations increased from Tanner 2 to 3 in girls and then declined during Tanner stages 4 and 5, with lower levels in girls than in boys (Fig. 1C). The boys had stable IGF-1 levels through the Tanner stages. IGF-1 (SDS) did not differ between the groups (see Table 1) but IGFBP-3 (SDS) was higher in index boys compared to controls (B = .31 [95% CI, 0.06-0.57], *P* = .02) (see Table 1). There was no difference in IGF-1 (SDS) and IGFBP-3 (SDS) between index and control girls.

In the path model, adjusting for pubertal stage and sex, IGF-1 (SDS) predicted lean mass (B = .008 [95% CI, 0.002-0.01], *P* = .01) in the entire cohort, which in turn predicted BMC/height (B = 19.6 [95% CI, 16.9-22.3], *P* < .0001) (Fig. 2). IGF-1 (SDS) predicted BMC/height adjusted for pubertal stage (B = .24 [95% CI, 0.02-0.45], *P* = .03) (see Fig. 2), but when testing lean mass as a mediator of this relationship, we found that the indirect effect of IGF-1 (SDS) on BMC/height was no longer significant (B = –.09 (–0.2 to 0.1), *P* = .60) (see Fig. 2). The path model was performed on the index group and controls separately. The findings were similar to the findings for the entire cohort with significant associations between IGF-1 and lean mass in each group, and in both groups the association between IGF-1 and BMC/height was mediated by lean mass (data not shown).

In the entire cohort, serum IGF-1 (SDS) was positively associated with insulin resistance (HOMA-IR) (B = .75 [95% CI, 0.37-1.12], *P* < .0001) and negatively associated with BIGTT-SI-0-30-120 (B = –.06 [95% CI, –0.08 to –0.03], *P* < .0001). IGF-1 (SDS) was positively associated with insulin secretion determined by BIGTT-AIR-0-30-120 (B = .59 [95% CI, 0.15-1.02], *P* = .009), reflecting that IGF-1 concentrations are negatively associated with insulin sensitivity and positively associated with insulin secretion. All analyses were adjusted for pubertal stage and sex. IGF-1 (SDS) was not associated with fat mass (adjusted for sex and pubertal stage) (B = .22 [95% CI, –0.15 to 0.07], *P* = .32). However, fat mass was associated both with BMC/height (B = 3.8 [95% CI, 2.9-4.7], *P* < .0001) and lean mass (B = 19.2 [95% CI, 16.7-21.7] *P* < .0001) (adjusted for pubertal stage and sex).

In the entire cohort, HOMA-IR was not significantly associated with BMC/height (B = –.30 [95% CI, –1.19 to 0.59], *P* = .51), and the results did not change when dividing the cohort according to sex. The moderating effect of the interaction between insulin resistance and IGF-1 (HOMA-IR×IGF-1) on lean mass was calculated (see Fig. 2). IGF-1 (SDS) and HOMA-IR (B = –.04 [95% CI, –0.08 to –0.01], *P* = .02) were both significantly associated with lean mass, but the interaction was not significant (B = .008 [95% CI, –0.02 to 0.03], *P* = .60), indicating that moderation was not present (Table 2). The moderating effect of HOMA-IR remained nonsignificant when dividing the cohort according to index and controls (data not shown).

Discussion

In this large cohort of offspring of mothers with T1D, we found no difference in serum IGF-1, bone mass, and muscle mass compared to controls, whereas fat mass was significantly increased among female offspring of women with T1D. In the entire cohort, we found a strong positive relation between IGF-1 and insulin resistance, but no effect of insulin resistance on bone mass. In contrast, IGF-1 levels were associated with BMC, and this association was

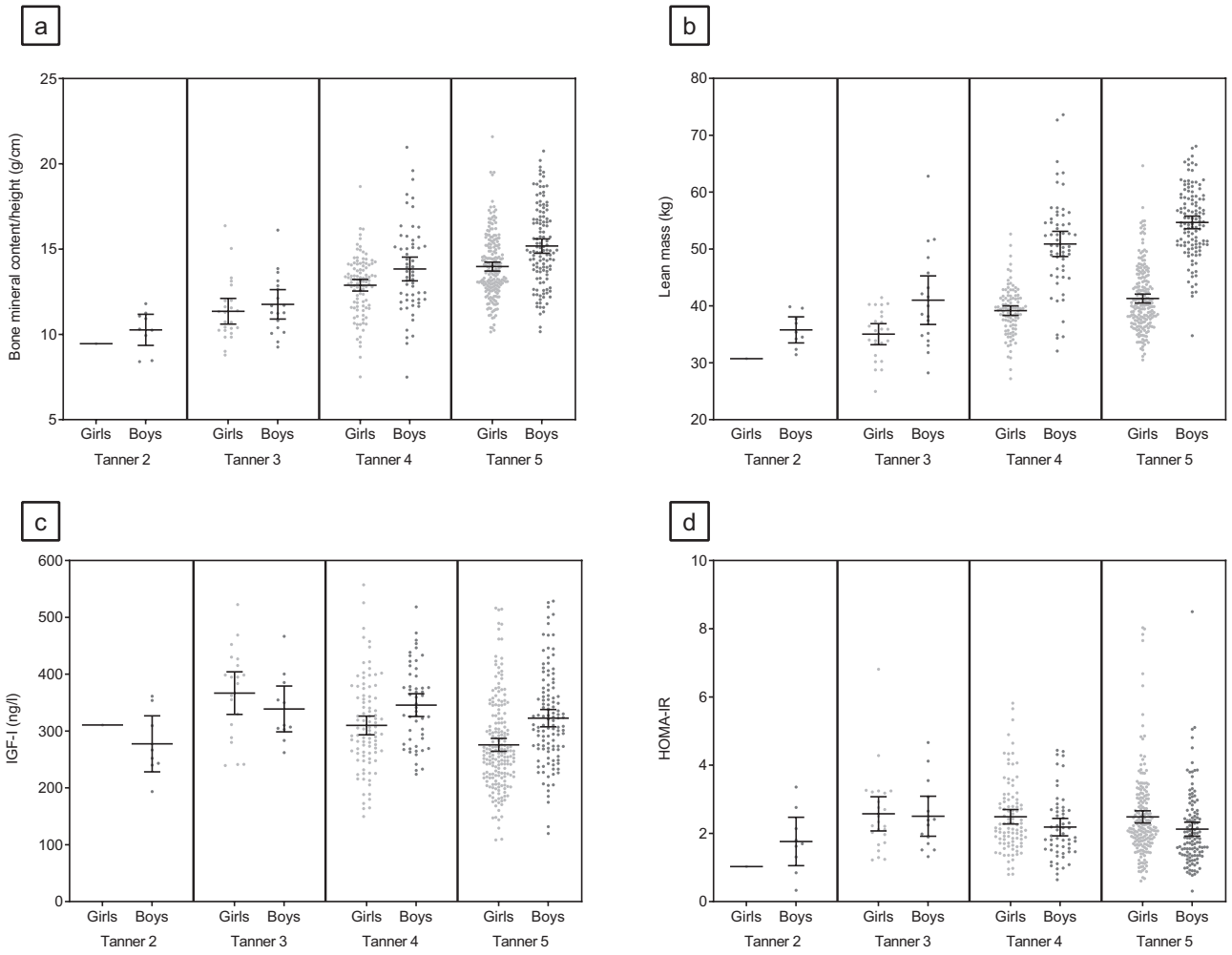

Figure 1. The cohort divided according to sex (girls: light gray dots, boys: dark gray dots) and Tanner stages 2 to 5: A, Bone mineral content/height (g/cm); B, lean mass (kg); C, insulin-like growth factor-1 (IGF-1) concentration (ng/L); and D, homeostatic model assessment of insulin resistance (HOMA-IR).

mediated by lean mass. In conclusion, lean mass was an intermediary factor in the IGF-1 bone relationship, which was not modulated by insulin resistance. Therefore, we can refute our primary hypothesis that insulin resistance negatively influences the muscle-dependent IGF-I–bone axis in neither the entire cohort nor when index cases and controls were analyzed separately.

During pubertal development transient insulin resistance follows the increased activity in the GH/IGF-I axis by an increase early in puberty, reaching the highest levels at Tanner stages 3 to 4 and decreasing thereafter (2, 3). The reduction in insulin sensitivity is more pronounced among girls, but can only partly be explained by increasing adiposity (1). In the present cohort of healthy adolescents, we found that IGF-1 levels especially among girls peaked at Tanner 3 and declined thereafter. Insulin resistance determined by HOMA-IR was stable throughout the pubertal stages both for boys and girls and did not follow the pattern of IGF-1. This may reflect that index cases and controls are both presented in the same figure. Our previous analyses on metabolism in this cohort showed that the difference in insulin sensitivity between the index cases and the controls increased with age (21), which thereby could explain the lack of decline in insulin resistance at Tanner 5 in the entire cohort. As reported previously, our cohort of offspring of mothers with T1D had a higher prevalence of components included in metabolic syndrome and prediabetes with reduced insulin sensitivity and relative insulin secretion deficiency compared with controls (20).

IGF-1 exerts anabolic effects on the skeleton by promoting osteoblastogenesis and inhibiting osteoblast apoptosis (29), increases osteoclastogenesis (30), bone resorption, and bone remodeling, and is key to acquisition of bone mass during adolescence (31). In the EPICOM cohort of adolescents, we found a significant correlation between IGF-1 and bone mass (BMC/height). However, in the causal path analysis, we found that this correlation disappeared when including lean mass, pointing toward lean mass as

Table 1. Anthropometrics, metabolic characteristics and body composition in index and control offspring

	Female		Male		Female			Male		
	Control (n = 182)	Index (n = 164)	Control (n = 121)	Index (n = 114)	Diff B	95% CI	*P*	Diff B	95% CI	*P*
Age, y	17.0 (1.7)	16.9 (1.6)	16.6 (1.6)	16.5 (1.6)	–0.21	(–0.6 to 1.6)	.27	–0.01	(–0.45 to 0.42)	.96
Weight, SDS	0.29 (1.1)	0.76 (1.4)	0.04 (1.1)	0.36 (1.1)	0.14	(0.22 to 0.78)	< .001	0.22	(–0.08 to 0.53)	.15
Height, SDS	–0.06 (0.9)	–0.24 (1.1)	0.25 (1.0)	0.39 (1.1)	–0.14	(–0.35 to 0.08)	.22	0.17	(–0.11 to 0.45)	.24
BMI, SDS	0.51 (1.1)	1.06 (1.3)	–0.09 (1.1)	0.24 (1.0)	0.55	(0.29 to 0.81)	< .001	0.22	(–0.07 to 0.52)	.15
IGF-1, SDS	–0.52 (0.8)	–0.45 (1.0)	–0.32 (0.8)	–0.14 (0.8)	0.06	(–0.13 to 0.25)	.54	0.18	(–0.06 to 0.41)	.14
IGFBP-3, SDS	0.34 (1.1)	0.40 (1.19)	0.38 (1.0)	0.65 (0.8)	0.09	(–0.16 to 0.34)	.48	0.31	(0.06 to 0.57)	.02
HOMA-IR	2.13 (1.8 to 3.0)	2.36 (1.7 to 3.1)	1.85 (1.4 to 2.4)	2.10 (1.5 to 2.7)	5.8	(1.6 to 10.0)	.007	4.7	(–1.5 to 10.5)	.12
BIGTT-IS	9.4 (3.4)	7.9 (3.6)	9.67 (3.0)	8.29 (2.9)	–1.6	–2.4 to –0.8)	< .001	–1.23	(–2.1 to –0.4)	.004
BIGTT-AIR	1791(1453 to 2198)	1992 (1573 to 2645)	1682 (1431 to 2082)	1838 (1503 to 2421)	0.05	(0.004 to 0.09)	.03	0.03	(–0.02 to 0.08)	.26
BMC, g	2234 (369)	2261 (390)	2539 (552)	2577 (517)	–9.4	(–84.7 to 65.8)	.81	30.1	(–98 to 158)	.64
BMD, g/cm^2	1.10 (0.1)	1.09 (0.1)	1.12 (0.1)	1.12 (0.1)	–0.01	(–0.03 to 0.01)	.29	–0.003	(–0.03 to 0.03)	.86
BMC/height	13.3 (2.0)	13.6 (2.0)	14.2 (2.7)	14.4 (2.6)	0.03	(–0.37 to 0.43)	.88	0.11	(–0.53 to 0.76)	.73
Fat, %	31.4 (27 to 35)	34.8 (29 to 40)	16.8 (13 to 22)	17.2 (15 to 25)	7.2	(3.9 to 10.5)	< .001	3.6	(–2.4 to 9.5%)	.24
Lean mass, kg	39.5 (37 to 43)	39.8 (36 to 43)	52.4 (45 to 57)	53.8 (49 to 58)	1.0	(1.1 to 1.3)	.83	0.9%	(–0.8 to 2.7)	.29

Data are presented as means (SD) or as medians (25th-75th percentile) if skewed distribution. Differences between groups are reported as estimates from linear regression B with 95% CI and *P* values. Data with skewed distributions are log-transformed and differences are given as percentage difference. All variables except age are adjusted for sexual maturation.

Abbreviations: BMC, bone mineral content; BMC/height, bone mineral content for height; BMD, bone mineral density; BMI, body mass index; BIGTT-AIR, oral glucose tolerance test–derived index of acute insulin response; BIGTT-IS, oral glucose tolerance test–derived index of insulin sensitivity; HOMA-IR, homeostatic model assessment of insulin resistance; IGF-1, insulin-like growth factor-1; IGFBP-3, insulin-like growth factor-binding protein 3; SDS, SD score.

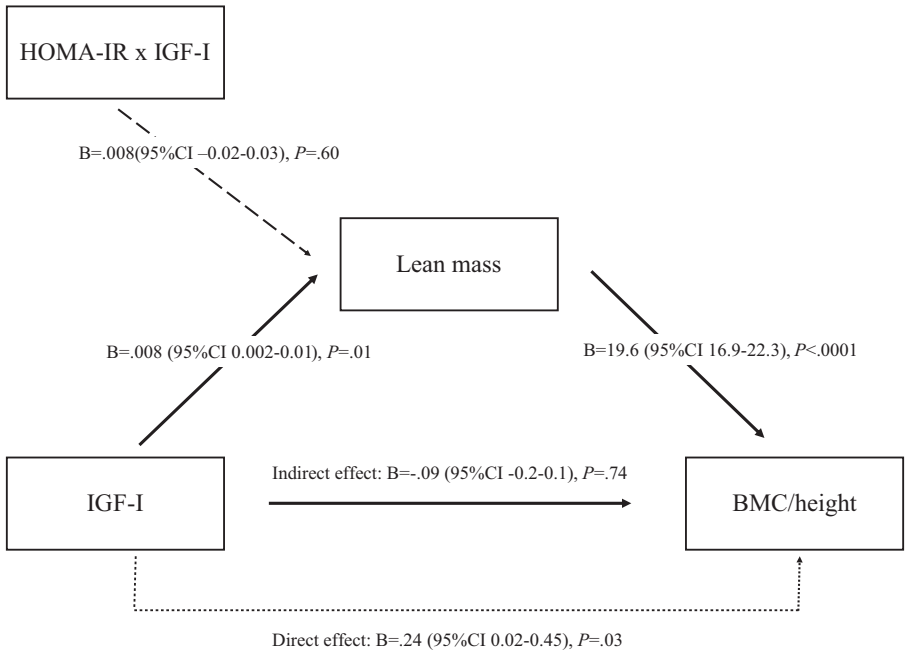

Figure 2. The dotted line represents the direct effect of insulin-like growth factor-1 (IGF-1) on bone mineral content for height (BMC/height) in the entire cohort. The solid lines represent the model with lean mass as a mediator of the relationship between IGF-1 and BMC/height. Unstandardized regression coefficients are presented as B (95% CI). All analyses include pubertal stage and sex as covariates. The broken line indicates the effect of the interaction between IGF-1 and homeostatic model assessment of insulin resistance (HOMA-IR) on lean mass.

Table 2. Linear regression analysis to determine the effect of interaction between insulin-like growth factor-1 and homeostatic model assessment of insulin resistance on lean mass

	B (95% CI)	SE	*P*
Constant	1.47 (1.42 to 1.52)	0.02	*P* < .0001
IGF-1, SDS	0.019 (0.01 to 0.03)	0.004	*P* < .0001
Log HOMA-IR	–0.04 (–0.08 to –0.01)	0.02	*P* = .02
IGF-1 (SDS)×log HOMA-IR	0.005 (–0.03 to 0.04)	0.02	*P* = .81

Data are reported as estimates from linear regression (B) with 95% CI, SE, and *P* values.

Abbreviations: HOMA-IR, homeostatic model assessment of insulin resistance; IGF-1, insulin-like growth factor-1; SDS, SD score.

an intermediary factor in the IGF-1–bone relationship in line with former studies (10, 11). A study of girls age 9 to 11 years by Kindler et al found that the HOMA-IR by IGF-1 interaction negatively predicted lean mass, and this moderating effect of HOMA-IR was stronger in participants with a better insulin sensitivity (HOMA-IR < 4.0) (11). When testing the moderating effect of the interaction between insulin resistance and IGF-1 on lean mass in the EPICOM cohort, we found there was no effect of insulin resistance, indicating that the effect of IGF-1 on skeletal muscle is independent of the sensitivity to insulin in adolescents. Our cohort was older and included both sexes compared to the cohort studied by Kindler and colleagues. Age and sexual maturation may play a significant role for this moderating effect in addition to sex, but our analysis of the moderating effect was still nonsignificant when dividing the cohort according to sex. Our present study confirmed the results of a study by Mughal et al, who examined 67 offspring of mothers with T1D with DEXA and pQCT) and found that they had higher bone area and BMC compared to controls (19). However, areal BMD and volumetric BMD did not differ between offspring and controls, indicating that the offspring had larger bones compared to the controls but the mineral content per unit area or volume did not differ. This cohort were smaller than our cohort but, in many ways, was comparable as the offspring had a higher BMI and a significantly higher fat percentage than controls, but this study did not include data on insulin sensitivity.

In the present study, insulin resistance and IGF-1 were positively associated in the entire cohort. Insulin promotes hepatic IGF-1 production, and additionally IGF-1 shares structural homology with insulin; they can bind the same receptors, but with major differences in affinity, and they share downstream signaling pathways (32). In studies of GH treatment of children born small for gestational age, we and others found that insulin resistance and IGF-1 levels were positively associated and that growth response during GH treatment was negatively associated with insulin resistance (33-35). In the EPICOM study we found no difference in height (SDS) between the index cases and controls. However, among the boys the

index cases had higher IGF-1 (SDS) and IGFBP-3 (SDS) levels compared to the controls, but only the difference in IGFBP-3 (SDS) reached significance. IGFBP-3 is 1 of 6 binding proteins that bind IGF-1 and thereby modulate IGF bioavailability. Traditionally, IGFBP-3 has been thought to be involved in metabolic regulation due to the binding of IGF-1, but recently studies have reported that IGFBP-3 may have a metabolic role independent of the IGF axis. However, the exact role of IGFBP-3 in glucose and lipid metabolism is still poorly understood. Overexpression of IGFBP-3 in transgenic mice resulted in fasting hyperglycemia, glucose intolerance, and insulin resistance (36), whereas data on IGFBP-3 knockout mice have been inconsistent. One study found that IGFBP-3 knockout mice who were fed a high-fat diet showed fasting hyperglycemia and hyperinsulinemia, indicating insulin resistance (37). Furthermore, in vitro studies have suggested that IGFBP-3 may lead to insulin resistance in adipocytes, and one study found that IGFBP-3 inhibited adipocyte differentiation (38). Adipocyte differentiation is required to mediate insulin sensitivity in adipocytes, and a possible inhibitory effect of IGFBP-3 on adipocyte differentiation could thereby lead to insulin resistance. Thus, the exact role of IGFBP-3 in metabolic regulation is not well understood, but it could be speculated that the increased concentrations of IGFBP-3 in the index boys in our cohort could play a role in adipocyte differentiation and thereby influence insulin sensitivity. Previously published results on adipokines from the EPICOM study showed that both male and female offspring of women with T1D had increased serum leptin and leptin/adiponectin ratio compared to controls, whereas serum adiponectin was reduced in females only. However, no direct association between maternal glycemic control during pregnancy and adiponectin and leptin levels or leptin/adiponectin ratio in the offspring was found (39).

Several studies have shown that obesity and insulin resistance during childhood may have a negative impact on bone mass and bone density (15, 16) and some reveal a sex difference (17), but many other studies find that overweight children have similar or even greater bone mass (13, 40). However, data are divergent and when adjusting for lean body mass, adiposity seems to be a negative predictor of bone mass during childhood (13, 40). However, in the present analyses we found that fat mass was positively associated with BMC/height even after adjusting for lean mass and pubertal stage. Detrimental effects on skeletal health in obese children may reflect a global health concern because the prevalence of childhood obesity is rapidly increasing worldwide. In addition, the EPICOM cohort represents a cohort of children whose mothers had T1D during pregnancy, which may have caused intrauterine hyperglycemia, hyperinsulinemia, and overgrowth. The effect of an adverse intrauterine environment on the risk of metabolic disease later in life is known as the *fetal programming effect*. In the EPICOM cohort we previously showed a programming effect on the metabolic risk as offspring of mothers with T1D had an adverse metabolic profile compared to controls (20, 21). However, the present data do not reveal a programming effect on skeletal health later in life, which confirms former studies suggesting birth weight and adult bone metabolism are unrelated when adjusting for size in adulthood (41).

A major strength of this study is that the cohort of adolescents is large and well characterized. We applied the statistical path analysis to explore the relationship between IGF-1 and bone mass and the modulating effect of insulin resistance. However, taking puberty into account a cross-sectional design is not optimal, and a longitudinal follow-up study through puberty would be necessary to explore the significant effects of puberty on the interaction between IGF-1, insulin sensitivity, and bone mass. Furthermore, the DEXA scan is a 2-dimensional measurement of bone mass, and a more thorough analysis of the microarchitecture of the bone using measurements such as pQCT would give more detailed information on the trabecular bone structure.

The findings in this cohort are considered to be generalizable to other cohorts of adolescents, but this cohort is homogeneous, consisting of White Danish children with a somewhat higher genetic height potential, which may theoretically affect the comparability of the cohort. However, we believe that taking height into consideration when evaluating bone mass will diminish this possible bias.

In conclusion, we here present that lean mass was an intermediary factor in the IGF-1 bone relationship in a large cohort of adolescents. We did not confirm previous findings in which the muscle-dependent relationship between IGF-1 and bone mass was found to be compromised by insulin resistance (11). The programming effect of a detrimental intrauterine environment was evident on insulin resistance and fat mass, but we found no difference in bone mass, IGF-1, or height between the adolescents exposed to T1D during pregnancy and the control participants. However, it may be speculated that the changes in insulin metabolism and adiposity in adolescence may have long-term harmful effects both on metabolic and skeletal health.

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Additional Information

Correspondence and Reprint Requests: Rikke Beck Jensen, MD, PhD, Department of Growth and Reproduction, Rigshospitalet, University of Copenhagen, Blegdamsvej 9, Copenhagen DK-2100, Denmark. E-mail: Rikke.beck.jensen@regionh.dk.

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