Birth size, body composition, and adrenal androgens as determinants of bone mineral density in mid-childhood

Running title: Birth size and bone mineral density

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Abstract

Background: Birth weight has an impact on adult bone mass. Higher birth weight is associated with greater bone mineral content (BMC) and children born small for gestational age (SGA) are at increased risk for impaired accrual of bone mass. Our aim was to study if the impact of birth size or early childhood growth on bone mass is visible already in mid-childhood.

Methods: We studied 49 children born large for gestational age (LGA), 56 children born appropriate for gestational age (AGA), and 23 children born SGA at 5.0–8.7 years of age. Body composition was assessed by whole-body dual-energy X-ray absorptiometry. Fasting blood samples and anthropometric data were collected.

Results: The children born SGA had lower bone mineral density (BMD) Z-score ($P<0.001$) and age- and sex-adjusted BMD ($P<0.005$) than the LGA and AGA children. Adjusted BMC, muscle mass, and body fat percentage (%BF) did not differ between the study groups. Muscle mass, BMI SD score (SDS), %BF, and serum dehydroepiandrosterone sulfate (DHEAS) concentration were the strongest predictors of high BMD in mid-childhood.

Conclusion: SGA-born children had lower BMD in mid-childhood compared to AGA- and LGA-born ones. Muscle mass or BMI SDS, %BF, and DHEAS were significant predictors of childhood BMD.

Introduction

Birth weight affects bone mass in adulthood, as higher birth weight is associated with greater bone mineral content (BMC) (1). Intrauterine growth restriction and programming are acknowledged to have an influence on cardiometabolic health in childhood and adulthood (2, 3), and children born small for gestational age (SGA), especially preterm, are at increased risk for impaired accrual of adult bone mass (1, 4, 5). Children born SGA without catch-up growth have lower total body bone mineral density (BMD) in early adulthood compared to those with catch-up growth (6).

Almost maximal bone mass is achieved during the first two decades of life (7). Nutrition, especially calcium and vitamin D, and mechanical load affect the final accrual (8). The association between BMI and volumetric BMD in children may mostly be determined by lean mass (LM) (9), not body fat mass that has been reported to have a negative association with bone mass in childhood (10, 11).

Vitamin D is essential for bone metabolism (12). Although serum 25-hydroxyvitamin D (25(OH)D) concentrations are recommended to exceed 50 nmol/l in children (13), there is evidence that low 25(OH)D status is common in children (14). The association between birth size and 25(OH)D concentrations has not been widely studied. No differences in serum 25(OH)D concentrations were detected between newborn infants born SGA, appropriate (AGA), and large for gestational age (LGA) (15), but it is unclear if the status is similar also in older children. In adults higher BMI leads to lower 25(OH)D (16) and the association of overweight with low 25(OH)D is seen also in children and adolescents (17).

The purpose of our study was to investigate whether the impact of birth size or early childhood growth on bone mass is visible already in prepubertal children.

Methods

The study cohort included 128 Caucasian children (67 boys) born singleton at term between 2004 and 2007 in Eastern Finland (18, 19). In brief, the children were enrolled according to their birth size and studied at 5.0–8.7 years of age [mean (95% CI, 6.9 (6.8–7.1) years] (Table 1). SGA was defined as gender-specific birth weight ≤ -2.0 SD score (SDS), LGA as birth weight $\geq +2.0$ SDS, and AGA as birth weight and length being between -1.0 and $+1.0$ SDS. Anthropometric data at birth, at the age of two years, and at examination were recorded.

BMI was calculated as the body weight divided by the square of the height (kg/m^2). Sex- and age-specific SDS for weight, height and BMI were calculated according to the recently published Finnish growth reference (20). Catch-up or catch-down growth was determined as an increase or decrease in weight or height SDS more than 0.67 during the first two years of life, respectively (21).

Areal BMD and BMC, body fat percentage (%BF), and muscle mass were assessed by whole-body dual-energy X-ray absorptiometry (DXA), using the Lunar device (Lunar Prodigy Advance; GE-Medical Systems, Madison, WI). BMD Z-scores were calculated using the recently published data by Crabtree et al. (22). LM was defined as a sum of muscle mass and BMC. Total body less head (TBLH) parameters were used for the analyses.

Fasting blood samples were collected for serum analyses of 25(OH)D, ionized calcium, dehydroepiandrosterone sulfate (DHEAS), and IGF-1 concentrations, and plasma analysis of alkaline phosphatase (ALP) concentrations. DHEAS Z-scores were created using recently published reference data on Caucasian children (23). Serum 25(OH)D concentrations were

assessed using chemiluminescence immunoassay (DiaSorin Inc., Stillwater, MN). Serum

ionized calcium and plasma ALP concentrations were determined with routine automated

methods (ion-selective electrode and photometric (The International Federation of Clinical

Chemistry and Laboratory Medicine (IFCC) recommendation), respectively) in the laboratory

of Kuopio University Hospital. Serum DHEAS concentrations were analyzed using

electrochemiluminescence immunoassay (Roche Diagnostics GmbH, Mannheim, Germany).

Serum IGF-1 concentrations were determined using an ELISA kit (Mediagnost, Reutlingen,

Germany).

A written informed consent was obtained from all parents and participating children aged ≥ 6

years. The study protocol was approved by the Committee on Research Ethics of the Hospital

District of Northern Savo.

Statistical analyses

Data are presented as mean (95% CI). Analyses were conducted using SPSS statistical

software (version 24; SPSS, IBM Corp., Armonk, NY). A significance level of 0.05 was used

in all analyses. Skewed data were either logarithm or square root transformed before

parametric analyses, and power transformed to geometric means for presentation. ANOVA

was used for comparisons between the study groups on anthropometric measures and BMD

Z-scores. ANCOVA was used for comparisons between the study groups on metabolic and

imaging parameters. Following factors were used for adjusting ANCOVA (vary between

analyses): month of the blood sampling, age, sex, BMI SDS, weight, and height. Obtained

estimated means are presented in Table 1. Linear regression was used to analyze factors

predicting bone mineral density. The regression coefficients are expressed as standardized

betas. Each model contained sex, age, 25(OH)D, ionized calcium, ALP, DHEAS, and IGF-1, and additional predictors were: Model 1: birth weight SDS, muscle mass, %BF; Model 2: birth length SDS, muscle mass, %BF; Model 3: the change in weight SDS from birth to the age of 2 years, muscle mass, %BF; Model 4: the change in height SDS from birth to the age of 2 years, muscle mass, %BF; Model 5: birth size group, muscle mass, %BF; Model 6: birth weight SDS, BMI SDS.

Results

Subject characteristics for the three birth weight groups are shown in Table 1. The SGA children had significantly lower age- and sex-adjusted BMD and BMD Z-scores compared to the LGA and AGA groups (Table 1). The boys in the whole study population had lower BMD than the girls [adjusted for age and height, P=0.028, mean (95% CI) 0.69 (0.68–0.70) and 0.70 (0.69–0.71) g/cm^2, respectively]. The boys had higher muscle mass [P=0.006, mean (95% CI) 16.8 (16.5–17.1) kg] and LM [P=0.011, mean (95% CI) 17.4 (17.0–17.7) kg] than the girls [16.1 (15.8–16.5) and 16.7 (16.4–17.1) kg, respectively]. The mean %BF of the boys was lower [P<0.001, mean (95% CI) 17.7 (16.0–19.5) than that of the girls 23.8 (21.5–26.5)] when adjusted for age and height. There were no statistical differences between the birth weight groups in BMC, muscle mass, LM, or %BF (Table 1). A significant difference was seen in plasma ALP concentrations between the groups (Table 1), but not between the sexes. The girls had significantly higher serum ionized calcium concentrations than the boys [P=0.001, mean (95% CI) 1.27 (1.26–1.28) and 1.25 (1.25–1.26) mmol/l, respectively]. A trend toward lower 25(OH)D concentrations (adjusted for the month of the blood sampling time, age, and BMI SDS) was seen in the LGA children compared to the AGA ones (Table 1, the post hoc test (Sidak correction) P=0.086).

Muscle mass or BMI SDS were the strongest weight-related predictors of higher BMD in the whole study population. Also, %BF was a significant predictor in the whole study population (Table 2). In the boys, being born SGA predicted lower BMD in mid-childhood (beta −0.27, P<0.001). Higher DHEAS concentrations predicted higher BMD in all models in the whole study population (Table 2, Figure 1) and among the boys (beta 0.22–0.26, P=0.001–0.006), but the association between BMD and DHEAS in girls remained weaker (beta 0.05–0.19,

P=0.053–0.548). The other biochemical parameters (25(OH)D, ionized calcium, ALP, IGF-1) did not significantly associate with BMD (Table 2).

Discussion

In this study, we evaluated the effect of birth size on BMD in prepubertal children. Being born SGA predicted lower BMD in prepubertal boys, but not in girls. Being born LGA had no significant impact on BMD. Current weight, especially muscle mass, was the strongest predictor of BMD in mid-childhood, but also birth weight SDS and serum DHEAS concentrations were positively associated with BMD.

There are not many studies on birth size and bone mass in childhood. In a recent cohort study, six-year-old SGA-born children had lower and LGA-born children higher BMC, but not BMD, compared to AGA-born ones (24). Biosca et al. showed a significant difference in age-, sex-, and weight-adjusted total body BMC between SGA (lowest mean), AGA, and LGA (highest mean) children at the age of 8 years, but when adjusted additionally for height the difference turned non-significant. No differences between the birth size groups were found in total body BMD (25). In a previous study on children aged 3–12 years, lumbar spine and femoral neck BMD were lower in SGA than AGA children (26). In our study, the SGA children had the lowest BMD among the study groups and lower birth weight SDS in the whole study population and being born SGA in the boys predicted lower BMD in mid-childhood. A positive association between birth weight and adult BMC, but not BMD, at the lumbar spine and hip has been reported previously (1). Children born SGA, especially preterm, are at increased risk for impaired accrual of adult bone mass (1, 4, 5).

Not only birth size, but also early growth has an impact on future bone mass. Catch-up growth especially during the first two years of life reduced the adverse effect of small birth size on childhood bone mass (24). SGA-born children had lower lumbar spine BMD Z-scores

than AGA-born children when there was no catch-up growth (26). Children born SGA with no catch-up growth had lower total body BMD in early adulthood compared to children born SGA with catch-up growth (6). Weight at the age of 1 year had a positive association with adult bone mass (1), and catch-up growth in weight but not in height predicted higher BMD in adulthood (6). Our data did not show any significant impact of early catch-up or catch-down growth in weight or height on BMD in mid-childhood.

The association between BMI and volumetric BMD in children may mostly be mediated by LM. It has been debated if fat mass is protective against fractures in childhood as it is in adults (27). Body fat mass is reported to have both positive and negative associations with bone mass in childhood (10, 11, 28). Our results did not show any differences in muscle mass, LM, or %BF between the birth size groups when adjusted for age, sex, and height. In linear regression analysis both high muscle mass and %BF predicted higher BMD in the whole study population.

Vitamin D is vital for bone metabolism by regulating calcium homeostasis, but it has also a positive effect on cardiometabolism (29), as serum 25(OH)D deficiency associates with cardiovascular disease (30), diabetes (31), and hypertension (32). Interestingly, vitamin D did not have a significant impact on BMD in our study cohort. Though, the LGA-born children did have a trend toward lower 25(OH)D concentrations than the AGA-born children. Other studies have showed an association between low vitamin D concentrations and low bone density in children and adolescents (33). In this study vitamin D concentrations were adequate in all groups, which may explain our result. Serum 25(OH)D concentrations are recommended to exceed 50 nmol/l in children for health benefits (13).

DHEAS is an adrenal androgen precursor and its concentrations increase individually through childhood (34). Children with premature adrenarche had higher areal BMD than their controls, but the differences were non-significant after adjusting for height SDS (35). We demonstrated recently that LGA children had lowest and SGA children highest DHEAS concentrations in mid-childhood (19). Linear regression analysis in the current study showed that DHEAS concentrations had a positive association with BMD in the whole study population and separately in boys but not in girls. DHEAS is metabolized to a more potent androgen testosterone and to estrone (36). There is evidence that both androgens and estrogens have a positive effect on bone mass accrual (36, 37). Our recent study showed a positive association between DHEAS and IGF-1 concentrations, but no differences in IGF-1 concentration were found between the birth size groups (19). IGF-1 has a significant impact on bone mineral accrual and it has been positively associated with BMC in prepubertal children (38, 39). In this study IGF-1 did not have any impact on BMD. This is in agreement with a previous report showing that IGF-I is a determinant of cortical bone mass but not cortical bone density (38).

We acknowledge several limitations in this study. First, the sample size was relatively small thus affecting the power of the analyses. Second, the AGA children were heavier than the Finnish children on average at examination. This might impact on the results, and that is why our analyses were adjusted also for body size. Third, the BMD was not measured as volumetric but areal. The International Society for Clinical Densitometry (ISCD) recommends TBLH DXA measurement as one of the methods for performing BMC and areal BMD measurements in pediatric subjects (40). The strengths of our study include the detailed data from birth, early childhood growth, and examination. The data at examination included anthropometric, biochemical, and imaging data. In addition, the study participants were

enrolled strictly according to the birth size, and the AGA group was selected to represent the children close to the mean birth weight and length.

In conclusion, children born SGA had lower BMD in mid-childhood compared to children born AGA and LGA. Muscle mass or BMI SDS were the strongest weight-related predictors of childhood BMD, while %BF and serum DHEAS as a marker of adrenal androgen secretion were also associated positively with BMD.

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Figure legend

Figure 1. Bone mineral density (BMD) mean Z-score (22) by tertile groups of dehydroepiandrosterone sulfate (DHEAS) Z-score (23) and body mass index SD score (BMI SDS) (20) of 120 prepubertal children.
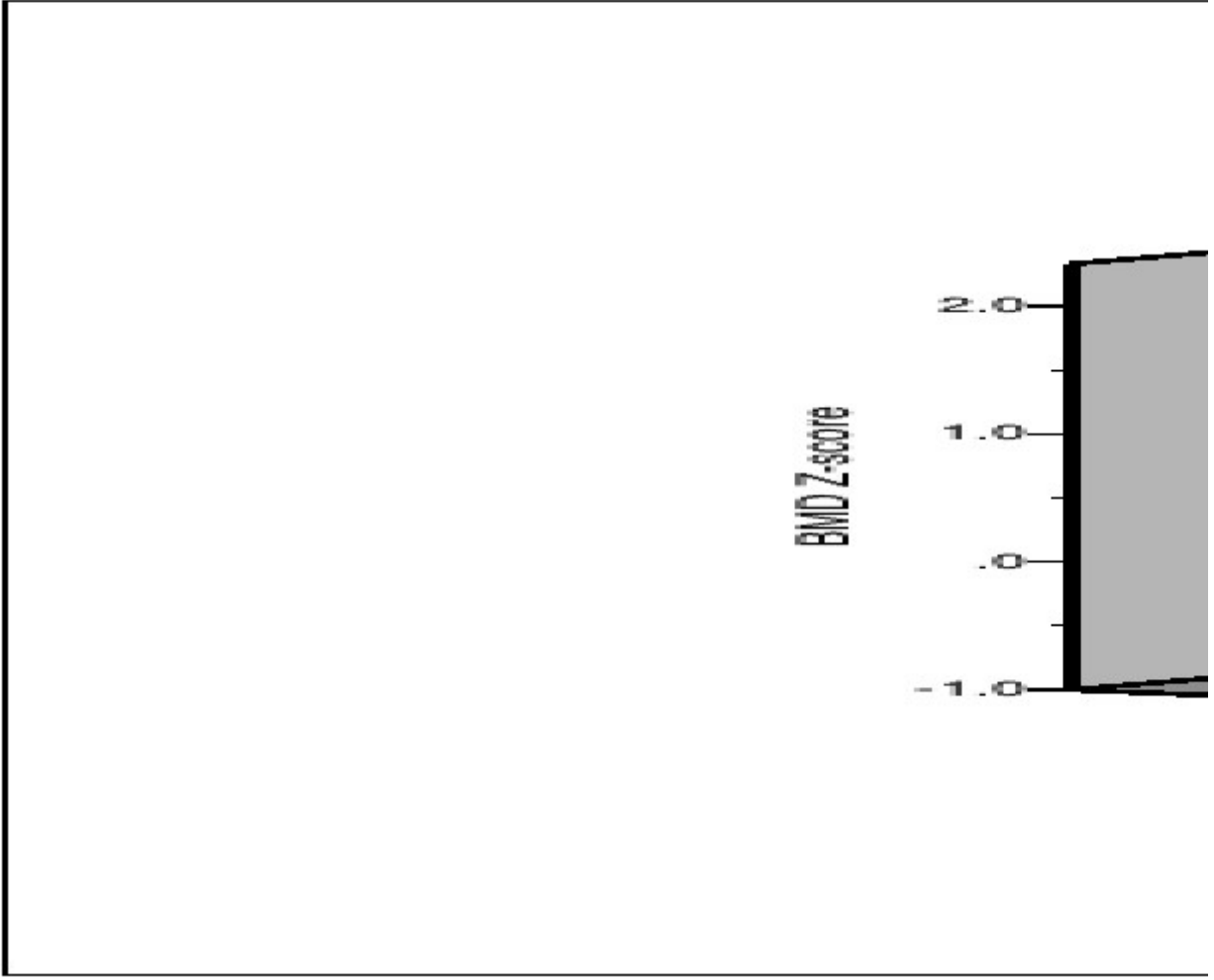
Table 1. Anthropometric, biochemical, and imaging characteristics of the study groups.

	LGA	AGA	SGA	*P*
Total number (boys)	49 (25)	56 (29)	23 (13)	
At birth				
Gestational age, weeks	39.8 (39.5–40.1)	39.9 (39.6–40.2)	39.7 (39.2–40.3)	0.810
Weight (g)	4722 (4631–4812)	3561 (3484–3637)	2476 (2345–2607)	<0.001
Weight (SDS)	2.63 (2.46–2.79)	−0.02 (−0.16–0.12)	−2.39 (−2.53– −2.25)	<0.001
Length (cm)	53.0 (52.6–53.4)	50.0 (49.7–50.4)	46.2 (45.5–46.9)	<0.001
Length (SDS)	1.58 (1.40–1.76)	−0.11 (-0.24–0.03)	−2.16 (−2.43– −1.88)	<0.001
At the age of 2 years				
Weight (SDS)	0.65 (0.41–0.90)	0.16 (-0.14–0.45)	-0.95 (-1.37– -0.53)	<0.001
Height (SDS)	0.40 (0.17–0.63)	-0.05 (-0.34 –0.24)	-0.98 (-1.33– -0.63)	<0.001
At examination				
Age (years)	6.89 (6.62–7.16)	7.09 (6.86–7.33)	6.65 (6.22–7.07)	0.130
Weight (kg)	27.6 (26.2–29.0)	27.5 (25.7–29.4)	21.8 (19.8–23.7)	<0.001
Weight (SDS)	0.68 (0.38–0.97)	0.39 (0.11–0.67)	−0.80 (−1.26– −0.33)	<0.001
Height (cm)	126.1 (124.0–128.3)	125.7 (123.8–127.7)	119.0 (115.6–122.4)	<0.001
Height (SDS)	0.54 (0.30–0.78)	0.20 (−0.07–0.46)	−0.64 (−1.01– −0.27)	<0.001
BMI (SDS)	0.56 (0.22–0.89)	0.36 (0.05–0.67)	−0.65 (−1.18– −0.12)	<0.001
25(OH)D (nmol/l)[a]	72.4 (68.0–77.2)	78.6 (73.9–83.7)	76.8 (70.3–83.9)	0.121
25(OH)D (nmol/l)[b]	73.5 (68.9–78.4)	80.5 (75.4–86.0)	74.5 (67.9–81.7)	0.082
Ionized calcium (mmol/l)[c]	1.27 (1.26–1.28)	1.26 (1.25–1.27)	1.27 (1.25–1.28)	0.217
ALP (U/l)[c]	230 (217-243)	251 (239–263)	228 (208–248)	0.038
DHEAS (μmol/l)[d]	0.49 (0.38–0.62)	0.67 (0.54–0.81)	0.83 (0.60–1.09)	0.028[d]
BMD (g/cm^2)[e]	0.70 (0.69–0.71)	0.70 (0.69–0.71)	0.66 (0.64–0.68)	0.002[e]
BMD (g/cm^2)[f]	0.69 (0.68–0.70)	0.70 (0.69–0.71)	0.68 (0.67–0.70)	0.242
BMD (g/cm^2)[g]	0.69 (0.68–0.70)	0.70 (0.69–0.71)	0.68 (0.67–0.70)	0.320
BMD (g/cm^2)[h]	0.70 (0.69–0.71)	0.70 (0.69–0.71)	0.68 (0.66–0.69)	0.068
BMD (Z-score)[i]	0.43 (0.13–0.74)	0.36 (0.08–0.63)	−0.65 (−1.15– −0.14)	<0.001[i]
BMC (g)[f]	589 (567–611)	599 (578–621)	561 (529–594)	0.163
Muscle mass (kg)[f]	16.7 (16.3–17.1)	16.4 (16.1–16.8)	16.0 (15.4–16.6)	0.154
Lean mass (kg)[f]	17.3 (16.9–17.7)	17.0 (16.7–17.4)	16.6 (16.0–17.2)	0.165
Body fat (%)[f]	21.0 (18.6–23.5)	21.1 (18.9–23.6)	18.6 (15.6–22.1)	0.454

Data are presented as mean (95% CI), except 25(OH)D, ionized calcium, DHEAS, BMC, and percent of body fat (geometric mean (95% CI)).

ANCOVA between the three study groups, except anthropometric data and BMD Z-score (ANOVA).

[a]Adjusted for the month of blood sampling.

[b]Adjusted for the month of blood sampling, age, and BMI SDS at examination.

[c]Adjusted for age and sex.

[d]Adjusted for age, sex, and BMI SDS.

[e]Adjusted for age and sex. The post hoc test (Sidak correction) between SGA and LGA/AGA *P*<0.005.

Table 2. Determinants of bone mineral density (total body less head) in the whole study population at examination (linear regression analysis).

	Model 1		Model 2		Model 3		Model 4		Model 5		Model 6	
P (Model)	<0.001		<0.001		<0.001		<0.001		<0.001		<0.001	
R square	0.78		0.78		0.79		0.79		0.79		0.70	
N	117		117		115		115		117		117	
	Beta	*P*	Beta	*P*	Beta	*P*	Beta	*P*	Beta	*P*	Beta	*P*
Female sex	0.10	0.071	0.11	0.065	0.11	0.049	0.13	0.026	0.10	0.088	0.02	0.679
SGA-born	-	-	-	-	-	-	-	-	−0.10	0.069	-	-
LGA-born	-	-	-	-	-	-	-	-	−0.04	0.501	-	-
Birth weight SDS	0.04	0.504	-	-	-	-	-	-			0.12	0.045
Birth length SDS	-	-	0.03	0.583	-	-	-	-	-	-	-	-
Δ Weight SDS 0–2 years	-	-	-	-	-0.07	0.133			-	-	-	-
Δ Length SDS 0–2 years	-	-	-	-	-	-	−0.08	0.086	-	-	-	-
Age	0.18	0.009	0.18	0.009	0.17	0.012	0.17	0.013	0.17	0.011	0.53	<0.001
Muscle mass	0.61	<0.001	0.61	<0.001	0.63	<0.001	0.63	<0.001	0.60	<0.001	-	-
Body fat percentage	0.18	<0.001	0.19	<0.001	0.19	<0.001	0.18	0.001	0.18	0.001	-	-
BMI SDS (at examination)	-	-	-	-	-	-	-	-	-	-	0.40	<0.001
25(OH)D	0.04	0.400	0.04	0.427	0.05	0.299	0.05	0.310	0.02	0.637	0.08	0.175
Ionized calcium	0.00	0.947	0.00	0.936	0.00	0.968	0.00	0.943	0.02	0.756	0.00	0.969
ALP	−0.03	0.489	−0.04	0.456	−0.03	0.508	−0.03	0.554	−0.05	0.300	−0.02	0.757
DHEAS	0.17	0.002	0.17	0.002	0.19	<0.001	0.18	<0.001	0.17	0.002	0.24	<0.001
IGF-1	0.05	0.389	0.05	0.390	0.05	0.394	0.05	0.396	0.04	0.416	0.11	0.083

SGA, small for gestational age; LGA, large for gestational age; SDS, standard deviation score; BMI, body mass index; 25(OH)D, 25-hydroxyvitamin D; ALP, alkaline phosphatase; DHEAS, dehydroepiandrosterone sulfate; IGF-1, insulin-like growth factor 1