CLINICAL STUDY

Serum markers of GH and insulin action in 12-year-old children born small for gestational age

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Abstract

Objectives: Our aim was to determine whether markers of growth hormone and insulin action differ between children born small for gestational age (SGA) and those born of an appropriate size for gestational age (AGA).

Design: Fifty-five SGA children and their 55 age- and sex-matched AGA control subjects were studied in a case-control setting at 12 years of age.

Methods: We examined serum concentrations of insulin-like growth factor (IGF)-I, IGF-II, IGF-binding protein (IGFBP)-1 and IGFBP-3, sex hormone binding globulin (SHBG), leptin, fasting insulin, and blood glucose. Insulin sensitivity was evaluated by the homeostasis model assessment for insulin resistance (HOMA-IR).

Results: The body mass index (BMI), sex, and puberty-adjusted mean serum IGF-I concentration was higher in the SGA than in the AGA children (303.4 vs 282.3 µg/l, $P = 0.006$). The mean serum concentrations of IGF-II, IGFBP-I, IGFBP-3, SHBG, fasting insulin, blood glucose and HOMA-IR did not differ between the SGA and the AGA group. The BMI, sex, and puberty-adjusted mean serum leptin concentration was lower in the SGA than in the AGA children (7.9 vs 10.1 µg/l, $P = 0.037$). In multiple logistic regression analysis, high HOMA-IR predicted high serum IGF-I levels in the SGA children (odds ratio 8.3; 95% confidence interval 1.7–41; $P = 0.010$), whereas in the AGA group HOMA-IR did not associate with the serum IGF-I level.

Conclusions: The BMI, sex, and puberty-adjusted mean serum IGF-I concentration was significantly higher and the leptin concentration was lower in the SGA than in the AGA children. No differences were found in the indices of insulin action or sensitivity between the SGA and AGA children at 12 years of age. However, HOMA-IR strongly associated with serum IGF-I levels in the SGA children.

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Introduction

Low birth weight has been associated with type-2 diabetes, cardiovascular diseases and also with short stature in later life (1–4). Approximately 10% of children born small for gestational age (SGA) are short as adults (adult height < -2 s.d. scores) (3, 4). Furthermore, intrauterine growth restriction (IUGR) may influence the growth hormone (GH) secretion profile in prepubertal children (5–7).

The insulin-like growth factors (IGFs) mediate many of the anabolic and mitogenic actions of GH. Serum levels of IGF-I and IGF binding protein (IGFBP)-3 reflect the endogenous GH secretion in healthy children; short children have lower IGF-I and IGFBP-3 levels than taller ones (8). During fetal life serum IGF-I levels are relatively low increasing with gestational age (9). In newborns, serum IGF-I levels correlate with birth weight and length; the IGF-I concentrations are lower in SGA than in appropriate for gestational age (AGA)

babies (9, 10). Serum IGF-I and IGFBP-3 concentrations increase slowly in early childhood with a steep increase during puberty, decreasing thereafter, while serum IGF-II concentrations are constant after the first few weeks of life (11, 12). Low birth weight has been associated with increased circulating concentrations of IGF-I in childhood (13–16). Cutfield and co-workers have reported that short IUGR born children have higher IGF-I and IGFBP-3 concentrations than their short AGA born control subjects at 7 to 8 years mean age (16). Furthermore, it has been hypothesized that abnormalities in somatotrophic actions of GH and IGF-I in short SGA children may contribute directly to reduced insulin sensitivity (7). IGFBP-1 is a specific IGF binding protein acutely modulating IGF bioactivity. Its expression in hepatocytes is downregulated by insulin, and serum IGFBP-1 concentration is a sensitive marker of insulin action (17). IGFBP-1 serum levels decline with age in prepubertal children, and the lowest values are found in puberty (12).

Leptin, produced by adipocytes, has a crucial role in the regulation of body fat mass, appetite and energy expenditure (18, 19). Leptin is detectable in fetal cord blood as early as 18 weeks of gestation, and at birth leptin concentration correlates with intrauterine growth (20, 21). In childhood, serum leptin levels increase with age, and girls tend to have higher levels than boys (22). In puberty, leptin levels decrease in boys, while the levels continue to increase in girls (22). In SGA children, leptin levels have been reported to be lower than in AGA children (23).

The aim of this study was to determine whether serum markers of GH (IGF-I, IGFBP-3) and insulin action (IGFBP-1, sex hormone binding globulin (SHBG)), insulin sensitivity (homeostasis model assessment for insulin resistance (HOMA-IR)), and leptin concentrations differ between SGA and AGA children in a population-based case-control setting.

Subjects and methods

Definitions

SGA was defined as birth weight or length or ponderal index > 2 s.d. scores below the respective mean for the gestational age (24). The ponderal index was calculated as $[\text{weight (g)}/\text{length}^3(\text{cm})] \times 100$. AGA was defined as birth weight, birth length and ponderal index > -2 s.d. scores and < 2 s.d. scores of the respective mean for the gestational age. Full-term was applied to babies born at or after week 37 and before the 42nd week of gestation (calculated from the beginning of the last menstruation). Body mass index (BMI) was defined according to the formula $\text{weight (kg)}/\text{height}^2$ (m). HOMA-IR was calculated as $[\text{serum fasting insulin (mU/l)} \times \text{blood glucose (mmol/l)}]/22.5$ (25).

Subjects

The study population consisted of all full-term children who were born SGA at Kuopio University Hospital, Finland, during a 22-month period between 1984 and 1986, 73 SGA children (70 singletons and three twins) were included in the study. Five 12-year-old children born SGA could not be reached, one child was excluded because of a metabolic disease, one child was excluded by age, and 11 were unwilling to participate in the study. Thus 18 (24.7%) subjects of the original SGA children were excluded from the study. Each SGA child had, as the control subject, the next born full-term AGA child matched for sex. At the age of 12 years, 55 SGA children (20 boys and 35 girls) and 55 AGA control subjects participated in this study. The mean age in both the study and the control group was 12.2 (s.d. 0.2) years. The study protocol was approved by the Research Ethics Committee of Kuopio University Hospital. Informed written consent was obtained from the child and the parents.

Methods

Perinatal data Perinatal data were obtained from hospital records. The birth measures were converted to s.d. scores by plotting them on the growth charts and adjusting the birth measures for duration of gestation and for gender (24). The mean birth weights and lengths for the SGA and AGA groups were 2452 g (-2.44 s.d. score) vs 3455 g (-0.24 s.d. score), $P < 0.001$, and 46.2 cm (-2.27 s.d. score) vs 50.4 cm (-0.08 s.d. score), $P < 0.001$ respectively. A detailed description of the perinatal characteristics has been reported previously (26). The mean birth measures of the children who dropped out of the study did not differ from those of the participating SGA children.

Anthropometric measures and pubertal development Height was measured in the 12-year-old children with a calibrated Harpenden stadiometer (Holtain Ltd, Crymych, Dyfed, UK) and recorded to the nearest 0.1 cm, and weight was recorded to the nearest 0.1 kg. Height was converted to s.d. scores and weight to percentages in relation to the mean weight-for-height by using the current Finnish reference values for height and weight-for-height (Pegasos V 3.9.2, Pediatric Research Foundation, Helsinki, Finland). A complete physical examination was performed on all children. The anthropometric measures at the age of 12 years in the SGA and AGA children are presented in Table 1. Pubertal development was assessed according to the Tanner staging scale (27, 28). For the statistical analyses, pubertal stage was defined by breast scores for girls and genital scores for boys (scores 1–5). Pubertal development did not differ between the SGA and AGA children at 12 years of age ($P = 0.917$, the marginal homogeneity test), even when the sexes were analyzed separately.

Laboratory methods Blood samples were taken in the morning, between 0900 h and 1000 h, after an overnight fast. An intravenous cannula was placed in the antecubital vein for blood sampling, and blood samples were drawn through the cannula. Serum IGF-I and

Table 1 Anthropometric data of the children born SGA and AGA at the age of 12 years. A more detailed description of the anthropometric data is described in a previous paper (26). Values are shown as the mean (s.d.).

Variable	SGA ($n = 55$)	AGA ($n = 55$)
Height (s.d. score)	-0.16 (0.90)***	$+0.60$ (1.00)
Height (cm)	150.1 (6.7)***	155.8 (7.5)
Weight for height (%)	-1 (17)*	$+9$ (22)
Weight (kg)	39.7 (7.6)***	48.6 (13.2)
Body mass index (kg/m^2)	17.5 (2.9)**	19.8 (4.2)

*** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$, SGA vs AGA (Wilcoxon matched-pairs signed rank test for differences).

IGF-II concentrations were analyzed by ELISA (DSL-10-5600 ACTIVE IGF-I ELISA; DSL-10-9100 ACTIVE IGF-II ELISA, both from Diagnostic Systems Laboratories, Inc., Webster, TX, USA). The assays include an extraction step in which IGFs are separated from their binding proteins in serum. The intra-assay coefficient of variation (CV) for IGF-I was 6.5%, and the interassay CV was 6.4%, as reported by the manufacturer; for IGF-II the respective coefficients of variation were 3.5% and 5.2%. Serum IGFBP-1 and IGFBP-3 concentrations were analyzed by ELISA (DSL-10-7800 ACTIVE Total IGFBP-1 ELISA; DSL-10-6600 ACTIVE IGFBP-3 ELISA, both from Diagnostic Systems Laboratories). The intra- and interassay coefficients of variation for IGFBP-1 were 2.5% and 6.8%, and for IGFBP-3 7.3% and 8.2% respectively. Serum leptin concentrations were analyzed by ELISA (Quantikine DLP00, R&D Systems, Inc., Minneapolis, MN, USA). The intra- and interassay coefficients of variation were 3.2% and 3.5% respectively. Serum SHBG was measured by the AutoDELFIA SHBG time-resolved fluoroimmunoassay (TR-FIA) method (Perkin Elmer Life Sciences Wallac, Turku, Finland). The intra- and interassay coefficients of variation were 4.0% and 2.6% respectively. Serum insulin concentrations were determined by RIA (Phadeseeph Insulin RIA, Pharmacia & Upjohn Diagnostics AB, Uppsala, Sweden). The intra- and interassay coefficients of variation for insulin were 5.3% and 7.6% respectively. Blood glucose concentrations were analyzed by a glucose oxidase method (Enzyme Electrode, Nova Biomedical, Waltham, MA, USA), and the respective coefficients of variation were 3% and 5%.

Data analyses

Data were analyzed using the statistical program SPSS for Windows, release 10.0 (SPSS Inc., Chicago, IL, USA). All continuous variables were examined for

normality with the Kolmogorov-Smirnov test. Non-normally distributed variables were log transformed before testing with parametric tests. Correlation coefficients were examined by Pearson's or Spearman's correlation tests. The Wilcoxon matched pairs signed rank test or paired sample *t*-test was used in comparing the means between the SGA and AGA group. Due to the skew distribution of serum leptin concentrations, median and range were reported in addition to the mean and s.d.. In comparing the differences between the sexes, the Mann-Whitney test was used. Serum markers of GH and insulin action were analyzed by paired sample *t*-test and repeated measures ANOVA adjusting for sex, difference of BMI, and puberty among the SGA-AGA pairs. Pubertal discrepancy was coded as -1, 0 or 1, when the SGA child had a lower, equal or higher score of pubertal development than the AGA control subject. Thus, discrepancy in pubertal development was included in the models as two dummy variables. If the repeated measures ANOVA gave a statistically significant result, double data of these comparisons were presented (Table 2). The factors associating with serum IGF-I level were studied by the multiple logistic regression analysis. A significance level of $P < 0.05$ was used for all analyses.

Results

Serum markers of GH action

Due to the differences in current weight, height and BMI between the SGA and AGA groups, the IGF-I concentrations were analyzed by adjusting for BMI, sex, and puberty. After adjusting for these variables, the mean serum IGF-I concentration was significantly higher in the SGA than in the AGA children (Table 2). Furthermore, IGF-I concentrations were higher in both the SGA and AGA girls, when compared with their

Table 2 The means (s.d.) of serum (S) IGF-I, IGF-II, IGFBP-1, IGFBP-3, fasting insulin, SHBG, leptin (the median and range) and blood (B) glucose concentrations and HOMA-IR at 12 years of age in the children born SGA and AGA.

Variable	SGA (<i>n</i> = 55)	AGA (<i>n</i> = 55)	<i>P</i> -value ^a
S-IGF-I (μg/l)	327.2 (126.2)	310.1 (127.6)	0.300
Girls	373.7 (118.3) ^b	351.5 (112.9) ^b	0.508
Boys	245.8 (96.1)	237.5 (121.6)	0.398
S-IGF-I (μg/l) ^c	303.4	282.3	0.006 ^c
S-IGF-II (μg/l)	849.3 (117.0)	835.3 (112.4)	0.535
S-IGFBP-3 (μg/l)	4763.5 (1566.0)	4269.3 (1368.8)	0.124
S-Insulin (mU/l)	9.8 (3.5)	10.6 (5.1)	0.617
B-Glucose (mmol/l)	4.4 (0.3)	4.3 (0.3)	0.558
HOMA-IR	1.91 (0.74)	2.03 (0.96)	0.694
S-IGFBP-1 (μg/l)	70.0 (34.8)	58.8 (30.3)	0.090
S-SHBG (nmol/l)	76.4 (30.3)	72.4 (35.9)	0.564
S-Leptin (μg/l)	12.7 (11.8)	18.7 (20.4)	0.233
	9.0 (0.8–50.7) ^d	9.4 (0.9–101.5) ^d	
S-Leptin (μg/l) ^c	7.9	10.1	0.037 ^c

^aPaired sample *t*-test for differences; ^bMann-Whitney test between the sexes, $P \leq 0.001$; ^crepeated measures ANOVA adjusted for BMI, sex, and puberty; estimated marginal means are presented; ^dmedian (range).

male peers. The means of IGF-II and IGFBP-3 concentrations did not differ between the SGA and AGA children (Table 2).

Serum markers of insulin action and leptin concentrations

The means of serum insulin and blood glucose concentrations and HOMA-IR did not differ between the SGA and AGA children, even after BMI, sex, and puberty were adjusted. Neither did the means of serum IGFBP-1 and SHBG concentrations differ between the SGA and AGA groups. After adjusting for BMI, sex, and pubertal development, the mean leptin concentration was lower in the SGA than in the AGA children (Table 2). In both the SGA and AGA group, HOMA-IR correlated positively with IGF-I (respectively $r = 0.525$, $P < 0.001$ and $r = 0.438$, $P = 0.001$), and with leptin levels (respectively $r = 0.384$, $P = 0.004$ and $r = 0.650$, $P < 0.001$), while serum IGFBP-1 correlated inversely with IGF-I (respectively $r = -0.285$, $P = 0.035$ and $r = -0.436$, $P = 0.001$), HOMA-IR (respectively $r = -0.496$, $P < 0.001$ and $r = -0.718$, $P < 0.001$) and leptin levels (respectively $r = -0.460$, $P < 0.001$ and $r = -0.609$, $P < 0.001$). HOMA-IR did not correlate with serum IGF-II concentrations.

Factors associated with high IGF-I levels in the SGA and AGA children

In the multiple logistic regression analyses, the IGF-I variable was dichotomized in the SGA and AGA groups; as the cut-off point we used the IGF-I level of the 75th percentile of the AGA group ($408.9 \mu\text{g/l}$). Sixteen (29.1%) SGA children passed this limit. According to the multiple logistic regression analysis, HOMA-IR associated strongly with a high serum IGF-I level in the SGA children. An increase of 1.0 unit in HOMA-IR increased by 8.3-fold the risk of a high serum IGF-I level. The other covariates in the model did not reach statistical significance (Table 3). Similar analyses were performed in the AGA children; none of the covariates reached statistical significance (data not shown). The SGA children in the highest IGF-I quartile of the AGA group ($n = 16$) had significantly higher BMI ($P = 0.021$), weight ($P = 0.038$) and weight-for-height ($P = 0.040$), and they tended to have lower

birth weight ($P = 0.077$) than the SGA children in the lower IGF-I quartiles ($n = 39$). Current height, expressed in s.d. scores, did not differ between these SGA subgroups ($P = 0.846$).

Discussion

We found that the BMI, sex, and puberty-adjusted mean serum IGF-I concentration was significantly higher, and the leptin concentration significantly lower in the 12-year-old SGA children than in their age- and sex-matched AGA control subjects. In the SGA children HOMA-IR associated with high circulating IGF-I levels in multiple logistic regression analysis.

The strengths of this study are its population-based sample and the case-control setting, in which the SGA children were matched by age and sex with their AGA control children. Although pubertal development did not differ between the SGA and AGA groups, we aimed to eliminate possible differences of pubertal development between the SGA-AGA pairs by adjusting it in the analyses. Furthermore, due to the significant differences in current weight, height, and BMI between the SGA and AGA children, we adjusted BMI in the analyses.

Serum IGF-I levels are age and puberty related (11, 29). In addition, current body composition and early catch-up growth may influence the circulating IGF-I concentration (14, 15). Ong and co-workers reported that IGF-I levels at 5 years of age are positively related to current weight and height, and to catch-up growth in weight or height between 0 and 2 years (15). Garnett and co-workers found that IGF-I levels in 7- to 8-year-old prepubertal children are inversely related to birth size, but positively related to current weight; consequently those who were smaller at birth but heavier at 7–8 years had the highest IGF-I levels (14). In our study, the circulating serum IGF-I concentrations were significantly higher in the SGA children than in their matched control subjects, when puberty, sex, and BMI were adjusted in the analysis. This finding is consistent with the recent reports showing that low birth weight has an inverse relationship with serum IGF-I levels (13–16). On the other hand, Woods *et al.* did not find any difference in IGF-I levels between prepubertal short SGA and short AGA children (7).

Table 3 Factors associating with high serum IGF-I concentration (the highest IGF-I quartile) in the SGA children ($n = 55$). Multiple logistic regression analysis.

Variables ¹	Regression coefficient	Significance	Odds ratio (OR)	Confidence interval for OR (95%)
HOMA-IR	2.12	0.010	8.3	1.7–41
Low birth weight (unit 100 g)	0.25	0.142	1.3	0.9–1.8
High BMI (kg/m^2)	0.18	0.255	1.2	0.9–1.6
Female sex	1.61	0.172	5.0	0.5–50

¹ Adjusted for pubertal stage.

Variation explained by the model: 57.0%.

However, in that study the SGA children were significantly shorter and leaner than their control subjects, and the comparison was not adjusted by current size. Furthermore, Cutfield *et al.* reported that IUGR children had higher IGF-I levels than short normal children, but lower levels than children with normal weight and height (16). In the present study, the SGA and AGA girls had significantly higher IGF-I concentrations than their male peers. A similar sex difference in prepubertal children has been reported previously (13, 14). In addition, girls reach their maximal IGF-I level earlier than boys - on average at 14.5 years of age and boys one year later (11).

Abnormalities in insulin sensitivity and glucose metabolism have been detected in childhood and early adulthood in individuals born SGA (7, 30–32). Recently, a hyperinsulinemic euglycemic clamp study revealed reduced insulin sensitivity in prepubertal SGA children (30). An intravenous glucose tolerance test showed higher early insulin response and lower insulin sensitivity in prepubertal short IUGR children than in short control children (31). Furthermore, higher fasting insulin levels, lower insulin sensitivity, and lower IGFBP-1 levels were found in 2- to 8-year-old SGA children when compared with normal birth weight controls (7). On the contrary, we found no differences in the means of fasting insulin, glucose or HOMA-IR between the SGA and AGA children, even though BMI, sex, and puberty were adjusted in the analyses. Possibly, fasting insulin levels and HOMA indices, the methods we used, were not sensitive enough to find differences between the groups. In addition, the SGA populations in different studies are not comparable; our sample was population-based, while some are selected to consist of short SGA children only. In the present study, neither the serum IGFBP-1 nor the SHBG levels, which have been suggested to be useful markers of insulin sensitivity (33), indicated decreased insulin sensitivity in the SGA children. HOMA-IR and fasting insulin levels correlated positively with IGF-I and inversely with serum IGFBP-1 concentrations in the SGA and AGA children. Moreover, in logistic regression analysis HOMA-IR was a significant factor associating with high circulating IGF-I levels in the SGA, but not in the AGA, group. The SGA children with higher current BMI and lower birth weight tended to be less insulin sensitive and to have higher IGF-I levels. In accordance with our findings, Cutfield *et al.* found a positive correlation between fasting insulin and IGF-I levels (16). On the other hand, in an adult study population high circulating concentrations of IGF-I were associated with a reduced risk for developing impaired glucose tolerance and type-2 diabetes (34). It has been suggested that IGF-I might have GH-independent effects on insulin sensitivity, and circulating IGF-I levels could have an important role in the regulation of insulin sensitivity and maintenance of insulin secretion (35).

We found that the BMI, sex, and puberty-adjusted serum leptin concentration was lower in the SGA than in the AGA group. This agrees with previous studies on leptin levels in SGA children. Woods *et al.* reported that short prepubertal SGA children have lower leptin levels than their short control children with normal birth weight (7). According to Boguszewski *et al.*, SGA children, with an age range of 2.0 to 12.8 years, had lower leptin levels than AGA children of the same age range (23).

In conclusion, the BMI, sex, and puberty-adjusted mean serum IGF-I concentration was significantly higher in the SGA than in the AGA children, which possibly reflects intrauterine programming of the GH–IGF-I axis and/or relative IGF-I resistance in SGA children. No differences were found in the indices of insulin action or sensitivity between the SGA and AGA children at 12 years of age. However, HOMA-IR strongly associated with serum IGF-I levels in the SGA children.

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