Vitamin D Levels and Insulin Resistance in Children Born with Severe Growth Restriction

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Abstract
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This study was designed to examine differences in serum 25(OH)D levels between small-for-gestational-age (SGA) and appropriate-for-gestational-age (AGA) prepubertal children in correlation with birth weight and indices of insulin resistance and β-cell function. Sixty-five nonobese children were examined at age 5–7.5 years; 27 born SGA and 38 matched AGA. Body weight, height, BMI, and waist circumference were recorded and fasting serum levels of glucose, insulin, 25(OH)D, and parathyroid hormone (PTH) were measured. The homeostasis model assessment for insulin resistance (HOMA-IR) and the β-cell function index (HOMA-β%) were estimated. The mean level of 25(OH)D was higher in the SGA group (26.2 ± 10 vs. 17.2 ± 7 ng/ml, $p < 0.01$) but that of PTH was no different. The insulin resistance and β-cell function indices were higher in the SGA group: HOMA-IR 1.34 ± 0.67 vs. 0.99 ± 0.53, and HOMA-β% 135 ± 56 vs. 97 ± 60 in the SGA and AGA groups, respectively. In the SGA group, 25(OH)D was correlated with HOMA-β% but not with HOMA-IR or insulin. In multiple regression, in the total cohort 25(OH)D and HOMA-IR were independently negatively correlated with birth weight ($\beta = -0.31$, $\beta = -0.36$, $p < 0.05$) respectively. In conclusion, at prepuberty severely in utero growth restricted children have increased birth weight dependent levels of 25(OH)D, which might exert a regulatory role on β-cell function.

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Introduction
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Recent studies have extended the activity of vitamin D [25(OH)D] well beyond that in calcium homeostasis and bone metabolism [1]. Clinical and experimental evidence supports a role of 25(OH)D in the pathogenesis of type 2 diabetes, with effects on either insulin sensitivity or β-cell function, or both [2,3]. Patients at risk for type 2 diabetes may have lower serum levels of 25(OH)D [3]. Positive correlation between levels of 25(OH)D and insulin sensitivity, and a negative effect of hypovitaminosis D on β-cell function have also been observed in persons without glucose intolerance [4,5].

Subjects born small-for-gestational age (SGA) because of intrauterine growth restriction constitute a population at risk of early development of chronic diseases, including type 2 diabetes [6]. A series of children born SGA had increased indices of insulin resistance early in life, correlated with both in utero growth restriction and accelerated postnatal growth [7–9]. 25(OH)D levels in correlation to insulin resistance have not been studied in this population. This case-control study was designed to investigate the relationships between serum levels of vitamin 25(OH)D, indices of insulin resistance and β-cell function, and birth weight in prepubertal children with severe in utero growth restriction.

Research Design and Methods
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The study aimed to include all children born SGA at the University Hospital of Ioannina after a term pregnancy (GA 37–41 weeks) during the 18-month period January 2003–June 2004. Most of the births (>80%) in a well-defined area of northwest Greece took place in this hospital. SGA children were defined as having birth weight <2 standard deviations (SDs) below the mean based on local growth charts. Of the children born SGA during the 18-month period, 44 were eligible; for 27 (15 males) of which the parents gave consent for participation in the study. Of the other chil-

dren, the parents of 12 did not wish their children to participate and for 5 the contact details were not found. The clinical and socio-economic characteristics of the nonparticipating children did not differ from those of the study group.

The control group comprised 38 children (19 males) born in the hospital during the same period, appropriate for gestational age (AGA), defined as having birth weight between the 20th and 80th percentiles for gestational age on local growth charts specific for age and gender. They were matched with the SGA children for age, gender, height, weight, body mass index (BMI), and pubertal status (stage 1 according to the Tanner criteria for puberty development). A total of 170 AGA children were initially contacted to indentify the 38 matched controls. All the study children were healthy, were not receiving drugs for any cause, and had no history of liver or renal disease or malabsorption. Exclusion criteria for both groups were: congenital malformations or genetic disorders, known metabolic disorder or chronic disease, obesity (BMI ≥95) at the time of the study, and a positive family history of diabetes or gestational diabetes.

The study protocol was approved by the Research Ethics Committee of Ioannina University Hospital. The parents of the eligible children were contacted, their written informed consent was secured and the children were evaluated at between 5 and 7.5 years of age. The following anthropometric data were recorded: birth weight, crown to heel length, and head circumference, obtained from the birth records, and body weight, body height, BMI and waist circumference at the time of the study, measured by standard methods. z-Scores for birth weight, BMI and waist circumference were derived from appropriate reference population standards. A morning venous blood sample for biochemical determinations was collected from each child after a rigorous 12 h fast, during the winter months (December–February); recruitment was balanced between SGA and AGA children across the months.

Fasting serum levels of glucose, insulin, 25(OH)D, and parathyroid hormone (PTH) were measured. Insulin was determined using an immunoenzymatic method (analyzer AXSYM, Abbott) and glucose by the glucose oxidase method. The homeostasis model assessment for insulin resistance (HOMA-IR) index and β-cell function (HOMA-β%) was used to detect the degree of insulin resistance and β-cell function, respectively [10]. HOMA-IR was assessed by the formula: [(insulin (mU/l)×glucose (mmol/l)]/22.5, and HOMA-β% by [20×insulin (mU/l)/(glucose (mmol/l)–3.5].

25(OH)D was determined by an enzyme-immunoassay (EIA) method using the kit of IDS Systems Ltd, UK. The sensitivity of the method was 5.0 nmol/l, and the intra- and interassay CVs were 5.3% and 4.6%, respectively. The biologically intact molecule of PTH (iPTH) was measured by a 2-site enzyme linked immunosorbent assay (ELISA) using the kit of BIOMERICA Inc. (USA). The sensitivity of the method was 0.09 pmol/l, and the intra- and interassay CVs were 3.2% and 7.7%, respectively. The sample volume required for each assay was 25 µl. A detailed questionnaire was completed by the parents of each child at the time of the study, concerning outdoor activities and food consumption, with emphasis on oral vitamin D preparations and foods containing or enriched with vitamin D (i.e., oily fish, eggs, and fortified breakfast cereals). Dietary vitamin D intake was estimated, based on the USDA food composition data (http://www.ars.usda.gov/nutrientdata), and on the labels in the case of the fortified breakfast cereals.

Statistical analysis

Students' t-test was used after examination of parameters for normal distribution. Simple and multiple regression analyses were conducted to define relationships among the examined parameters, namely 25(OH)D level, birth weight z-scores, insulin resistance and β-cell function indices, BMI, waist circumference z-scores and age. A sample size of 65 children could define a 25% difference among parameters with a power of 80% at the p < 0.05 level [11]. Statistical analyses were performed using the Stat View software package of SAS Institute Inc. (Cary, USA).

Results

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According to the questionnaire data, the AGA and SGA children showed no differences in their diet (caloric content, food type, etc.) or time spent on outdoor activity. The mean dietary vitamin D intake estimated on a weekly basis was no different between the 2 groups: 990±250 (SGA) and 950±235 (AGA) IU/week.

As shown in ◉ Table 1, the mean serum level of 25(OH)D was higher in the SGA group. In 2 SGA and 20 AGA children, the level of 25(OH)D was below a cutoff value of 15 ng/ml, while in 3 of the AGA children it was below 10 ng/ml. Only 6 SGA and 2 AGA children had 25(OH)D levels above 30 ng/ml, which has recently been proposed as the cutoff of adequacy.

The insulin resistance indices for fasting insulin and HOMA-IR were higher in the SGA group, while HOMA-β%, which represents β-cell function, was favorable in this group. PTH level did not differ between the 2 groups. By comparing children with 25(OH)D levels below and above 15 ng/ml no differences in insulin resistance and β-cell function indices were found (data not shown).

In simple regression analysis in the SGA group, the 25-OH D level was positively correlated with HOMA-β% (R=0.38, p < 0.05, ◉ Fig. 1), but not with HOMA-IR or fasting insulin (R=0.16, 0.14, respectively, p=ns). In the AGA group 25-OH D was not correlated with either HOMA-β% or HOMA-IR or insulin or (R=0.17, −0.08, −0.07, respectively). PTH showed no correlation with vthe metabolic indices in any group or the total cohort.

Table 1 Anthropometric characteristics and metabolic variables in prepubertal children, born small (SGA) or appropriate (AGA) for gestational age.

|	SGA (n=27)	AGA (n=38)
At birth		
Gestational age (weeks)	38.4±1.3	38.3±1.3
Birth weight (g)	1 894±440***	3 338±390
Birth weight (g) z-score	−3.42±92***	−0.13±0.98
Birth length (cm)	45.1±3.7**	51.3±2.6
At the time of study		
Age (years)	5.9±1.6	6.2±1.1
Body weight (kg)	25.2±7.1	26.7±6.1
Body height (cm)	119±9	120.8±9
BMI (kg/m²)	17.4±3.1	17.8±2.1
BMI-SDS	−0.08±1.0	0.29±0.7
Waist circumference-SDS	0.04±1.2	0.53±1.2
25(OH)D (ng/ml)	26.2±10***	17.2±7
iPTH (pg/ml)	21.8±14	23.2±9.2
Fasting glucose (mg/dl)	80.2±9.2	82.3±6.5
Fasting insulin (µIU/ml)	6.35±3.4*	4.62±2.21
HOMA-IR	1.34±0.67*	0.99±0.53
HOMA-β%	135±56*	97.0±60

*p<0.05; **p<0.01; ***p<0.001
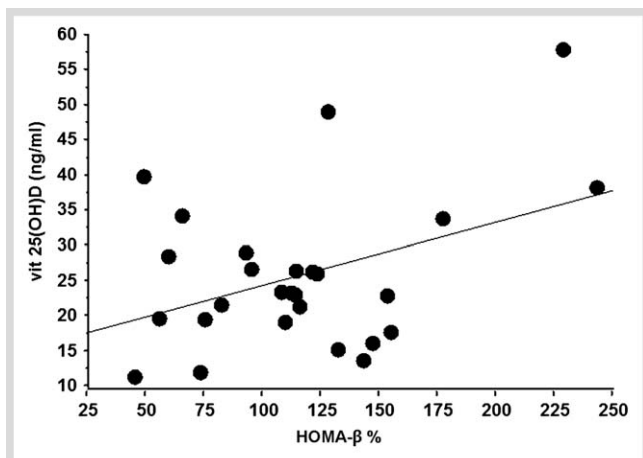
Fig. 1 Relationship between serum 25(OH)D level and HOMA-β% in the small for gestational age (SGA) children of the study (n=27).
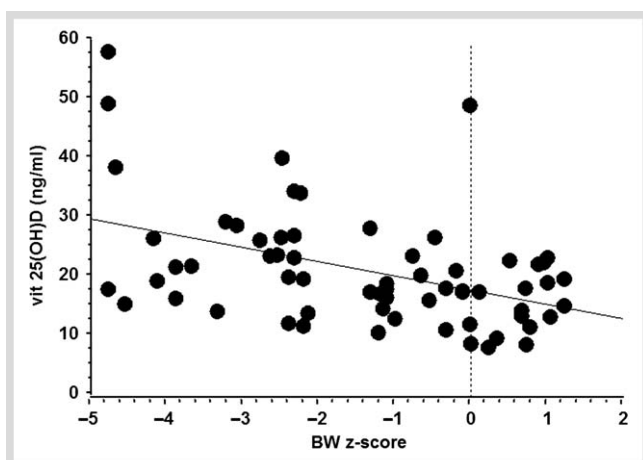

Fig. 2 Relationship between serum 25(OH)D level and birth weight (BW) z-scores in the study children (n=65).

Multiple regression analysis was conducted for the total cohort of children to identify possible relationships between the birth weight z-score (dependent variable) and 25(OH)D levels, insulin resistance indices, and obesity indices after appropriate adjustments. The birth weight z-score, was negatively associated with 25(OH)D ($\beta = -0.31$, $p=0.02$) after adjusting for waist circumference, BMI, HOMA-IR, and age (**Fig. 2**). The birth weight z-score was negatively associated with HOMA-IR ($\beta = -0.36$, $p=0.003$) after adjusting for BMI, waist circumference z-scores, 25(OH)D, and age. This relationship was stronger without 25(OH)D in the model ($\beta = -0.39$, $p<0.0004$) but continued to be strong after entering 25(OH)D. Finally the birth weight z-score was positively associated with waist circumference z-score ($\beta = +0.28$ $p=0.03$) after adjusting for HOMA-IR, insulin, 25(OH)D, and age.

Discussion
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This study showed that children born severely SGA had higher levels of 25(OH)D than those of AGA in prepuberty, affected indices of insulin resistance (insulin and HOMA-IR) in accordance with the findings of other researchers [7–9] but improved insulin secretion (HOMA-β%). No correlation between 25(OH)D and insulin resistance was found but a positive relationship

between 25(OH)D and HOMA-β% was demonstrated in the SGA group.
Studies conducted mainly in adults have shown inverse relationships between 25(OH)D levels and indices of insulin resistance or risk of type 2 diabetes or several other components of the metabolic syndrome and positive relationships between 25(OH)D levels and β-cell function [4, 5, 12–14], although these findings were not confirmed in some reports [15]. Conversely, in agreement with the present study, several studies in children have failed to show association between vitamin D and insulin resistance [16–20]. Even in studies in children where correlation between vitamin D and several components of the metabolic syndrome was found, independent relationships between insulin resistance indices and 25(OH)D were not confirmed [21–23]. Pacifico et al. [23] found that after making appropriate adjustments (including BMI) only blood pressure and the metabolic syndrome remained significantly correlated with vitamin D, but not with IR indices (insulin or HOMA). Similarly, Gannagé-Yared et al. [22] showed that vitamin D was an independent predictor of systolic blood pressure and fasting glucose, but not of insulin or HOMA-IR.
It could be speculated that at this early period of life other factors may influence vitamin D and insulin resistance. One such factor could be PTH, as vitamin D deficiency results in hyperparathyroidism [24, 25], through which glucose metabolism may be affected. Higher PTH, but not lower vitamin D, was shown to increase the risk of metabolic syndrome in a recent study [26]. In the present study PTH levels were similar in the 2 groups, implying that the observed difference in vitamin D may not be sufficient to affect the PTH level. Furthermore, no clear association between PTH level and insulin resistance indices was found.
Another explanation is that the levels of vitamin D, although higher in the SGA group, were below the recently proposed insufficiency threshold (32 ng/ml), thus lacking a favorable effect on the insulin resistance, which could possibly confer a higher 25(OH)D serum level. A Canadian study in children showed that even a high increment in 25(OH)D level (i.e., 10 ng/ml) correlated with only a slight decrease in the fasting blood glucose level and HOMA-IR [18]. There is also some evidence for a lower threshold by which vitamin D deficiency confers negative effects on insulin sensitivity [27]. In a previous study in adolescents, this threshold was estimated at 15 ng/ml [27], but application of this threshold to the present study generated no differences in insulin resistance indices.
Other studies have demonstrated correlation of high 25(OH)D levels with β-cell function rather than insulin resistance [28]. This was the case in the present study in SGA group where the higher, positively correlated with 25(OH)D, HOMA-β% imply increased β-cell function. The higher HOMA-β% may attenuate the risk for impaired glucose tolerance posed by the higher HOMA-IR in this group, as the risk of the 2 parameters is regarded as additive [29].
The higher vitamin D levels in SGA children are difficult to interpret, but they could be related to differences in adipose tissue between the 2 groups. Although the groups were matched for BMI, waist circumference was about 0.5 z-score higher in AGA group. This difference, although not statistically significant may be physiologically significant with regard to vitamin D serum level. Higher adiposity values would provide a larger distribution volume and lower circulating 25(OH)D in the AGA group. Higher 25(OH)D level was shown to be independently correlated with low birth-weight in the present study. This relationship

may arise from alterations in adipose tissue due to in utero growth restriction. It has been demonstrated that SGA children have a diminished capacity to store fat subcutaneously (the metabolic equivalent of a partial lipodystrophy) [30], thus the SGA children in this study may have had a limited overall storage capacity for vitamin D, with the result that higher circulating vitamin D levels were measured. This hypothesis, however, is difficult to prove, as fat partitioning and tissue vitamin D were not studied in the present study.

Catch-up growth occurs in over 90% of SGA children. This was the case for the SGA study children, who were all within the normal range of development at prepuberty, and around the mean, despite their severe growth restriction at birth. The increased insulin resistance indices may therefore be related with both their low birth-weight and subsequent accelerated growth [7,8]. Obese children were excluded from the present study to avoid the well documented effect of adiposity on both 25(OH)D level and insulin resistance status, thus the results of the present study are limited in nonobese SGA children who had experienced catch-up growth.

Other limitations of this study are the small sample size and the use of surrogates of glucose homeostasis, although they correlate well with standard methods [10,31]. The small number of included children could lead to sample bias limiting the possibilities of finding other associations beyond those already indentified. Moreover possible selection bias could not be excluded since the final number of included SGA children was considerably smaller than the original target population. The duration of sunshine exposure was based on parental recall, so slight differences in time spent outdoors could confound the estimations of vitamin D status. The differences in 25(OH)D levels were too large, however, to be attributed to slight deviations in sun-exposure time between the 2 groups belonging to the same cohort and living in the same geographical area (latitude 39° N). Finally, as the study was observational, the alterations found in the parameters measured may be physiologically unrelated or due to other unrecognized factors.

To the best of authors' knowledge, this is the first study to examine 25(OH)D in a special population with severe in utero growth restriction and a tendency to become insulin resistant early in life. Nonobese SGA born children had higher serum 25(OH)D levels, which were correlated with insulin secretion, but not with insulin resistance indices. Low birth weight appears to be a factor that is correlated independently with both 25(OH)D levels and insulin resistance in this group of children.

Acknowledgements
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Conflict of Interest
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