Reduction of IGF-binding protein-3 as a potential marker of intra-uterine growth restriction

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Abstract

Background: Growth factor-binding proteins influence the growth of infants starting *in utero*. Adaptation of the fetus to an adverse uterine environment is associated with changes in the growth hormone-growth factor-insulin axis.
Aims: To evaluate serum levels of IGF-I and IGFBP-3 in small and appropriate for gestational age newborn infants.
Methods: Fifty-four newborn infants, small (SGA, n=28) or appropriate (AGA, n=26) for gestational age were matched by gestational age and sex. Blood was collected on the first day of life, and anthropometric measurements were taken at birth. The serum levels of IGF-I and IGFBP-3 were compared, and correlated with the anthropometric measurements.
Results: On the first day of life, mean serum IGFBP-3 levels were significantly lower in SGA babies and correlated with weight, length, head circumference, and ponderal index (weight/length 3) (P<0.0001). In contrast, no associations were found between IGF-I serum levels and these anthropometric measurements.
Conclusion: Our data show that SGA babies have significantly reduced IGFBP-3 concentrations at birth.

Keywords: IGF-binding protein-3; insulin-like growth factor-I; small for gestational age.

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Introduction

Insulin growth factors have a central role in fetal-placental growth throughout gestation. It has been shown that insulin-like growth factor (IGF)-I levels at birth are lower in small for gestational age (SGA) than in appropriate for gestational age (AGA) preterm newborns [10, 20], although other studies evaluating only term SGA newborns found higher IGF-I levels [29, 32] than in AGA infants. Changes in the hormonal milieu may be a consequence of the adaptation of the fetus during intrauterine life to a hostile environment of maternal, placental or fetal origin in order to improve survival. Many studies have shown that this adaptation has consequences either in the neonatal period or later [8, 14, 26]; for example, there is the high risk of developing insulin resistance at the end of childhood or as young adults for SGA and premature children [6, 11, 12, 21, 33]. The endocrine re-programming that occurs in this population is the result of modifications in the growth hormone-growth factor-insulin axis during intra-uterine life and is influenced by nutrition in the first post-natal weeks [1, 28].

In addition, circulating IGF-1 forms complexes with binding proteins (IGFBPs), whose physiological functions include transport, reservoir, protection from proteolytic degradation, and the ability to activate receptors on target cells [27]. Therefore, serum IGFBPs, especially IGFBP-3 [15, 30], are important markers of growth.

In this study, we evaluated the serum levels of IGF-I and IGFBP-3 in SGA and AGA newborn infants at birth, matched by gestational age and sex, to test the hypothesis that intrauterine growth restriction is associated with these hormones early in life.

Methods

All SGA newborn infants born between 1 July 2005 and 31 June 2006 at the Instituto Fernandes Figueira maternity, Rio de Janeiro, Brazil, were included in the study. SGA newborn infants were matched with AGA newborn infants, born in the

same period and unit, by gestational age and sex. The exclusion criteria were congenital malformations, congenital infections and genetic syndromes.

SGA was defined as birth weight ≤ 2 SD (standard deviation) below the mean (weight for age Z-score, ≤ -2 SD) using the Kramer's reference data [16], a restricted definition by the international consensus published by Lee et al. [19]. The children with birth weights above the cut-off were considered AGA. Gestational age was calculated from the last menstrual period (40), early ultrasound (9), or the New Ballard score (5), in that order of preference.

Anthropometric measurements were taken at birth by trained nurses. Weight was measured with a digital scale (precision of 5 g). An appropriate scale for an incubator was used for length measurements and an inextensible tape was used for head circumference measurements. Blood samples were taken on the first day of life.

Serum IGF-I and IGFBP-3 levels were measured in the Laboratório de Fisiologia da Nutrição e do Desenvolvimento, Departamento de Ciências Fisiológicas, IBRAG, Universidade do Estado do Rio de Janeiro, by radioimmuno assay (IRMA™ Active IGFBP-3 DSL-6600 and IRMA™ Active IGF-1 DSL-5600). The IGF-I extraction was done with an HCl ethanol solution. An anti-IGF-I antibody marked with I-125 was used for IGF-I measurements. The coefficients of variation were 3.0 for intra-assay (mean = 52.5 ng/mL; SD = 16.4) and 1.5 for the inter-assay (mean = 53.8 ng/mL; SD = 8.0) differences. An anti-IGFBP-3 polyclonal goat antibody marked with I-125 was used for IGFBP-3 measurements. The coefficients of variation were 3.2 for the intra-assay (mean = 27.23 ng/mL; SD = 1.4) and 1.5 for the inter-assay (mean = 21.9 ng/mL; SD = 0.1) differences.

Differences in proportions were evaluated using the χ^2-test with Yates correction. A non-parametric test (Wilcoxon test) was used for comparisons of the levels of IGF-I and IGFBP-3 between the groups, and a Spearman correlation was used to analyze the relationship between these factors and birth weight, head circumference, length at birth and ponderal index. All analyses used the SAS (Statistic Analysis System, version 9.01) software. The study was approved by the Ethics Committee of the Institution, and one of the parents signed a written informed consent.

Results

The mothers of 57 children were invited to participate, and 54 (94.7%) agreed. Due to paired inclusion, 28 were SGA (17 preterm) and 26 were AGA (15 preterm). The demographic characteristics of the population are shown in Table 1. There were no significant differences between the two groups at birth, except for birth weight, birth length, head circumference and weight for age Z-score (P < 0.0001).

On the first day of life, mean serum levels of IGFBP-3 but not of IGF-I were significantly lower in SGA babies (Table 2). There were borderline correlations between IGF-I and birth weight (0.28, P = 0.048) and IGF-I and the ponderal index (0.27, P = 0.06). However, there were no correlations between IGF-I and length at birth, head circumference at birth or gestational age (P-values of 0.12, 0.41 and 0.22, respectively). IGFBP-3 had a strong, positive correlation with birth weight (0.67, P < 0.0001), birth length (0.64, P < 0.0001), head circumference (0.60, P < 0.0001), ponderal index (0.69, P < 0.0001) and gestational age (0.44, P = 0.001).

Discussion

In this study, no significant difference was found in the IGF-I levels between SGA and AGA newborn infants on the first day of life, although IGFBP-3 levels were lower in SGA infants. This lack of difference between SGA and

Table 1 Characteristics at birth of the small for gestational age (SGA) and appropriate for gestational age (AGA) newborn infants.

Variable	SGA (28) Mean (SD)	AGA (26) Mean (SD)	P-value
Maternal age, years	25.7 (6.8)	27.1 (9.8)	0.54
Gestational age, weeks	35.0 (3.5)	35.0 (3.6)	0.96
Weight, g	1500 (599)	2437 (716)	<0.0001
Length, cm	41.0 (5.5)	47.4 (4.7)	<0.0001
Head circumference, cm	29.0 (3.3)	32.2 (2.5)	<0.0001
	n (%)	n (%)	
Sex, male	8/28	7/26	0.86
Premature*	17/28	15/26	0.96
Multiple gestation	5/28	6/26	0.89
Cesarean section	25/28	17/24	0.15
APGAR (5 min) ≤ 6	4/28	1/26	0.19
Smokers	5/28	4/25	0.57
Alcohol use	1/22	7/23	0.04
Maternal hypertension	16/27	9/25	0.16
Oligodramnious	12/26	6/24	0.20
Premature rupture of membranes	2/23	5/25	0.41

*14/54 (25.6%) gestational age <33 weeks.
SD = standard deviation, SGA = small for gestational age, AGA = appropriate for gestational age.

Table 2   Means, SD and 95% CI of IGF-I and IGFBP-3 serum levels for SGA and AGA newborn infants in the first day of life.

Variable	SGA (26) Mean (SD) 95% CI	AGA (25) Mean (SD) 95% CI	P-value
IGF-I (ng/dL)	2.78 (2.37) 1.82–3.74	3.99 (3.04) 2.73–5.24	0.17
IGFBP-3 (ng/dL)	541.9 (295.9) 455.4–628.5	682.4 (287.1) 597.2–767.6	0.007

Wilcoxon test.
SD = standard deviation, CI = confidence interval, SGA = small for gestational age, AGA = appropriate for gestational age.

matched controls indicates the important role of gestational age in IGF-I changes. This observation is in agreement with a previous study performed with fetal blood samples that showed a progressive increase in IGF-I levels with gestational age; by comparing the IGF-I levels of small (below the mean weight for gestational age) and large (above the mean weight for gestational age) fetuses, the authors found lower levels in the small fetus, although the difference between the groups was not significant up to 33 weeks of gestation [18]. The infants included in the present study are a heterogeneous population in relation to gestational age, as they were selected as SGA independent of gestational age, as were their counterparts: about 60% in each group were preterm, and 25.9% of the entire population had a gestational age of <33 weeks.

Levels of IGF-1 for gestational age reported in fetal samples throughout gestation have shown an increase toward the end of gestation [9, 17, 18]. There are also reports of IGF-I levels in cord blood [2, 22, 25, 30, 32] and a decrease in IGF-I levels from cord blood to the first and fourth days of life has been observed [10, 23, 29]. However, Giudice et al. (1995) obtained different results, showing that IGF-I levels were unchanged in preterm newborn infants, whereas in term neonates IGF-I levels decreased on day one, remained low during the first three days of life, and then increased to birth levels by the end of the first week [10]. In our study, blood was collected on the first day of life from babies of different gestational ages due to the matching by gestational age and sex. Consequently, our IGF-I and IGFBP-3 values are not comparable to those reported in the literature because they represent mean values from preterm and full-term neonates.

On the other hand, the lack of difference in IGF-I levels between SGA and AGA infants on the first day of life could be the effect of attenuation since approximately one fourth of the babies had a gestational age of <33 weeks, where a difference in IGF-I levels between SGA and AGA infants would not be expected. IGFBP-3 levels have no correlation with gestational age [18] and, therefore, were not influenced by the prevalence of preterm babies in this sample born at <33 weeks. Only two previous reports of higher IGF-I levels in SGA newborn infants [29, 32] and one report of a lack of difference in IGF-I levels between AGA and SGA newborn infants [25] were found.

In a study considering constitutional growth potential based on maternal characteristics, Mamelle et al. (2006) demonstrated that the group of infants classically classified as SGA was heterogeneous in respect to having suffered *in utero* growth restriction: some were SGA by weight and/or length but were in fact not growth restricted [24]. Additionally, some infants, classified as AGA by weight and/or length, had suffered growth restriction [24]. This could also happen in our population, confounding the results of IGF-I on the first day of life. Verkauskiene et al. (2007) showed that cord IGF-I and IGFBP-3 concentrations were significantly decreased in growth-restricted infants, defined by serial ultrasound measurements during gestation, in both AGA and SGA babies [31]. Their study used two classifications to define growth restriction: 1) a reduction of >20% of the estimated fetal weight from the 22nd gestational week until birth, based on serial ultrasound scans, and 2) a classification at birth as AGA or SGA using the 10th percentile of a customized growth chart as a cut-off. Similar to Mamelle et al. (2006) [24], Verkauskiene et al. (2007) found AGA babies who were growth restricted and SGA babies who were not growth restricted. In our study, we tried to minimize this difficulty by using a more restricted cut-off to define SGA: birth weight equal to or <2 SD below the mean. In conclusion, the correct classification of growth restriction *in utero* is extremely important for understanding its effect on the hormonal status of the newborn infant.

Although this study did not reveal differences in the levels of IGF-I between the two groups of infants in the first day of life, IGFBP-3 levels were lower in SGA babies and strongly correlated with weight, length, head circumference and ponderal index, demonstrating its role in fetal growth here and in other studies [7, 13]. The apparent disassociation between IGF-I and IGFBP-3 levels in this particular situation may be explained by the actions of IGFBP-3 [3, 4]. IGFBP-3 is the most abundant IGFBP circulating in human serum, has both inhibitory and stimulatory effects in cell systems, and modulates growth. IGFBP-3 can prevent IGF-I from binding to its receptors since IGF-I has a higher affinity to IGFBP-3 than to the IGF-I receptor, thus having an inhibitory action on growth. IGFBP-3 also functions as a reservoir of IGF-I, carrying it to target cells [4]. In addition, IGFBP-3 not only carries IGF-I to the target organs, but also activates IGF-I receptors on target cells. However, IGFBP-3 alone

has no direct stimulatory effect; it acts through activation of IGF-I receptors. It has been demonstrated that IGFBP-3 increases the bioeffectiveness of IGF-I by 2- to 4-fold [5]. Therefore, the equal levels of IGF-I in both AGA and SGA babies and lower levels of IGFBP-3 in SGA babies may indicate that action of IGF-I is diminished by IGFBP-3 reduction, resulting in the growth restriction of children.

In conclusion, this study demonstrates that IGFBP-3 plays an important role in promoting fetal growth and is a good marker for evaluating *in utero* growth. However, the classification of *in utero* growth restriction is fundamental to documenting this evidence. It is important to identify these children at birth because they could be at risk for metabolic diseases later in life.

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