Evaluation of IGF1/IGFBP3 Molar Ratio as an Effective Tool for Assessing the Safety of GH Therapy in Small-for-Gestational-Age, GH-Deficient and Prader-Willi Children

Short title: IGF1/IGFBP3 Molar Ratio and rGH Safety

Mériem Gaddas 1*, Laurence Périn 2, Yves Le Bouc 3
1 Department of Physiology and Functional Explorations, Faculty of medicine « Ibn el Jazzar », University of Sousse, Tunisia.
2 Assistance publique - Hôpitaux de Paris (AP-HP), Trousseau Hospital, Department of Paediatric Endocrinology, 75012, Paris, France.
3 Sorbonne Université, INSERM, Centre de Recherche St-Antoine UMR S938, AP-HP, Trousseau Hospital, Department of Paediatric Endocrinology, 75012, Paris, France.

What is already known on this topic?
GH therapy is widely used, but concerns have been raised that it might increase cancer and cardiovascular risks.

What this study adds?
This study provides support for the use of IGF1/IGFBP3 molar ratio as a tool for assessing the safety of the therapeutic adaptation of GH therapy in children.

Corresponding author:
Mériem Gaddas, Department of Physiology and Functional Explorations, Faculty of medicine « Ibn el Jazzar », University of Sousse, Tunisia
+0021698569921
meriem-gaddas@hotmail.fr
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https://orcid.org/0000-0002-8836-767X

Abstract
Objective: IGF1 concentration is the most widely used parameter for the monitoring and therapeutic adaptation of recombinant human growth hormone (rGH) treatment. However, more than half the variation of the therapeutic response is accounted for by variability in the serum concentrations of IGF1 and IGFBP3. We therefore compared the use of IGF1/IGFBP3 molar ratio with that of IGF1 concentration alone.

Methods: We selected 92 children on rGH for this study and assigned them to three groups on the basis of growth deficiency etiology: small for gestational age (SGA), GH deficient (GHD) and Prader-Willi syndrome (PWS). Plasma IGF1 and IGFBP3 concentrations and their molar ratio were determined.

Results: Before rGH treatment, mean IGF1/IGFBP3 molar ratio in the SGA, GHD and PWS groups was 0.14 ± 0.04; 0.07 ± 0.01 and 0.12 ± 0.02, respectively. After the initiation of rGH treatment, these averages were 0.19 ± 0.07, 0.20 ± 0.08 and 0.19 ± 0.09, within the normal range for most children, even at puberty and despite some significant increases in serum IGF1 levels.

Conclusions: We consider IGF1/IGFBP3 molar ratio to be a useful additional parameter for assessing therapeutic safety on rGH, keeping values within the normal range for age and pubertal stage.

Keywords: GH therapy, IGF1/IGFBP3 molar ratio, Growth Hormone Deficiency, Small for Gestational Age, Prader-Willi Syndrome

Introduction
IGF1 concentration remains the most widely used parameter for the monitoring and adjustment of recombinant human growth hormone (rGH) treatment (1). However, more than 58% of the variation in the therapeutic response to rGH over the first year of treatment in children can be explained by the variability of serum concentrations of IGF1 and IGFBP3 (2).

In current practice, total IGF1 concentration is measured, without the determination of free IGF1 levels due to technical difficulties (2). Free IGF1 can be assumed as the bioactive form, but it is in equilibrium with the IGF1 engaged in large and small complexes according to the mass action law; almost 99% of the IGF1 in serum is engaged in such large and small complexes (1).

It is therefore impossible to determine tissue bioavailability and, thus therapeutic efficacy or safety, from IGF1 or IGFBP3 assays alone. Furthermore, a lack of correlation between daily GH secretion and total IGF1 concentration has been reported in some cases (1). This situation may be due to the presence of various polymorphisms affecting sensitivity to GH, which may differ for IGF1 and IGFBP3. It should also be stressed that IGF1 concentrations vary considerably between individuals (3,4).
Finally, the dynamics of serum concentrations for IGF1 and its carrier protein, IGFBP3, remain unclear in patients on rGH treatment, and conflicting results have often been obtained (5). Moreover, high IGF1 concentrations have been implicated in cancer, whereas IGFBP3 has a protective effect (6). We therefore believe that determining the bioavailability of IGF1 by calculating the IGF1/IGFBP3 molar ratio would provide more information about the safety of GHR treatment than the use of total IGF1 assays alone.

Methods
This retrospective study was performed at the Hormonology and Functional Endocrine Explorations Laboratory of Armand Trousseau Hospital in Paris. We selected, from our database, 92 children on rGH treatment followed at our outpatient clinics. These children were assigned to three groups on the basis of the etiology of their growth deficiency: 20 children who were small for gestational age (SGA), 61 children with GH deficiency (GHD group), and 11 children with Prader-Willi Syndrome (PWS) (see Table 1a). The retrospective study of the patients' files that were exploited covered the period 2011-2017, and the clinical and biological data under treatment correspond to the period March 2016-March 2017. The duration of the treatments and the follow-up of the patients vary from 2 to 7 years. The doses of rGH administered were as follows: 34.96 ±14.35 µg/kg/day for the SGA group, 25.56 ±10.01 µg/kg/day for the GHD group and 21.67 ± 8.45 µg/kg/day for the PWS group. No adverse events attributable to rGH were reported in these children during follow-up.

Clinical (etiologic diagnosis, height, weight, BMI, rGH dose) and biological (IGF1, IGFBP3, insulin, fasting glycemia, lipid status, HbA1C) data were collected by consulting the patient's medical records. Clinical parameters (height, weight and BMI) were standardized relative to the corresponding national reference ranges (7). IGF1 and IGFBP3 are expressed as Z-scores (SDS) adjusted for age, sex and pubertal stage. Baseline clinical and biological characteristics were obtained for each of the etiological groups (Table 1a). National ethics and confidentiality rules were respected, in accordance with the legislation in force for retrospective studies of cohort files.

The IGF1 and IGFBP3 assays were performed with the IDS-Isys system, in an automated procedure based on ELISA with detection by chemiluminescence. The IDS-Isys device was calibrated according to the new WHO international standard for IGF-I NIBSC 02/254, in accordance with the recommendations of the Growth Hormone Research Society and the International IGF Research Society (8, 9, 10, 11).

Serum IGF1 and IGFBP3 concentrations were interpreted by comparison with the reference intervals established specifically for this ISIS device by Bidlingmaier et al. (10) for IGF-I and by Friedrich et al. (11) for IGFBP3. The IGF1/IGFBP3 molar ratio was calculated according to the formula as previously described (11, 12): 

\[
\text{IGF1/IGFBP3 molar ratio} = \frac{\text{IGF1 (ng/ml) } \times 0.13}{\text{IGFBP-3 (ng/ml) } \times 0.035}
\]

The reference values used for the interpretation of IGF1/IGFBP3 molar ratios were based on the data collected in our pediatric functional endocrine exploration department at Trousseau Hospital (see Table 1b). These data come from a control population made up of children who came to consult for follow-up of unrelated pathologies: moderate well-balanced asthma follow-up under inhaled corticosteroid, isolated hypospadias or testicular ectopy, moderate growth retardation of around -1SDS and well-balanced hypothyroid children. These data were obtained from control children of different ages and pubertal stages and were comparable, as described by Friedrich et al. (11), for IGF1/IGFBP3 molar ratio determinations.

The study design (retrospective analysis of the data) was approved by the Ethical Committee of Trousseau hospital without an approval number. Informed consent has been obtained from the parents after full explanation of the purpose and nature of all procedures used.

Statistical Analysis
ANOVA was performed to compare quantitative and qualitative variables between groups. A paired t-test was performed to compare qualitative variables between groups. Differences were considered significant if the P value was <0.05. A correlation analysis (spearman) was carried out between the individual values of the 3 ratio and those of quantitative parameters.

Results
Before rGH treatment, the mean molar ratio was 0.14 ± 0.04 (range: 0.09-0.23) in the SGA group, 0.07 ± 0.01 (range: 0.06-0.08) in the GHD group and 0.12 ± 0.02 (range: 0.10-0.14) in the PWS group (Figure 1a).

The IGF1/IGFBP3 molar ratio values of the various groups in this study improved on rGH treatment, reaching values in the normal range for healthy children of the same age (Table 1a and 1b). Mean IGF1/IGFBP3 molar ratio (± SD) on rGH treatment was 0.19 ± 0.07 (range: 0.12-0.35) for the SGA group, 0.20 ± 0.08 (range 0.04-0.36) for the GHD group and 0.19 ± 0.09 (range: 0.04 -0.32) for the PWS group (Figure 1b). There was a positive correlation between the individual dose values of rGH and the individual values of the whole ratio (p = 0.02); however the correlation between low-normal and high ratio groups and growth increments is not significant (p = 0.15), probably due to the heterogeneity of age and duration of treatment.

In the SGA group (Figure 2), two children with high serum IGF1 concentrations (> + 2 SDS) had normal IGF1/IGFBP3 molar ratios, because their IGFBP3 levels were also high (> + 2 SDS).

In the GHD group, an analysis of IGF1 concentration according to age and pubertal stage after treatment revealed that 13% of children had IGF1 concentrations > + 2 SDS, particularly during the pubertal period. However, when IGFBP3 levels were also taken into account, the IGF1/IGFBP3 molar ratios of these children were found to be in the normal range for age and pubertal stage (Figure 3 and table 1b). We also found one five-year-old child with a normal IGF1 concentration but a very high molar ratio (> + 2 SDS), because of a very low IGFBP3 concentration (<2 SDS), requiring therapeutic adaptation (Fig. 3).
We investigated the potential utility of the IGF1/IGFBP3 molar ratio for use in the adaptation of rGH treatment according to the "quartiles" of Park and Cohen (29). Indeed, these representations of the risk of cardiovascular ischemia for IGFBP3 concentrations initially described by the histograms of Juul et al. (28) and subsequently confirmed by the studies of Cohen, highlight the need to take both IGF1 and IGFBP3 into account (28), because these two parameters have "opposite" biological actions (28) and a dynamic "yin-yang" relationship (29).

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These findings reflect the U-shaped correlation curve obtained for the relationship between serum IGF1 and IGFBP3 concentrations initially described by the histograms of Juul et al. (28) and subsequently confirmed by the studies of Cohen, highlight the need to take both IGF1 and IGFBP3 into account (28), because these two parameters have "opposite" biological actions (28) and a dynamic "yin-yang" relationship (29).

We investigated the potential utility of the IGF1/IGFBP3 molar ratio for use in the adaptation of rGH treatment in three different etiological groups (children SGA, or with GHD or PWS). Indeed, SGA children are characterized by "GH resistance ", resulting in a need for higher rGH doses than are used in other children matched for age, sex and puberty stage (5, 30). In children, rGH dose is generally adjusted progressively, on a case-by-case basis (31). There are currently no clear guidelines on the mode of therapeutic adaptation according to the results obtained (12, 29). This complicates therapeutic management, because it is considered important to keep IGF1 concentration below + 2 SDS (31).

By contrast, almost all children with PWS children have a somatotropic deficit (32, 33), justifying the systematic early initiation of rGH treatment, within the first year of life (32, 33, 35). Unlike SGA children, children with PWS are particularly "rGH-sensitive" and have very high serum IGF1 concentrations (towards and above the upper limit of the normal range) (33, 34, 36).

For the three pathological entities studied (GHD, SGA and PWS), we found that the determination of IGF1/IGFBP3 molar ratio was a useful additional tool for the adaptation of rGH treatment with a view to improving safety.
The safety of rGH treatment is generally ensured by monitoring to keep serum IGF1 concentration at values below the + 2 SDS threshold for age and pubertal stage (12). Our results indicate that there may be discrepancies between total IGF1 concentration and IGF1/IGFBP3 molar ratio with a potential impact on the safety of rGH treatment. The use of this ratio made it possible to optimize rGH administration so as to minimize the risk of adverse effects, particular those of a metabolic, cardiovascular or neoplastic nature (17, 28, 37).

Calculation of the IGF1/IGFBP3 molar ratio is, thus, a potentially useful additional tool because this ratio does not necessarily vary with increases in IGF1 concentration and it takes into account the variation of serum IGFBP3 levels (12).

We were able to determine approximate values for IGF1/IGFBP3 molar ratio before and after the initiation of rGH treatment in children SGA or with GHD or PWS. We found that the mean ratio increased after the initiation of rGH treatment in all groups, but that it remained within the reference range for age and pubertal stage in most children. Our values were consistent with those reported in previous studies. Romer et al. (38) reported a low ratio in patients with GHD (0.13 ± 0.07) before rGH, with a sharp increase to more than 0.32 ± 0.07 after treatment initiation. Our results are also consistent with those of Cabrol et al. (12), who reported a mean molar ratio before treatment of 0.14 (range: 0.10 to 0.27) in children SGA, increasing to 0.19 (range: 0.15 to 0.23) after treatment.

Finally, on rGH treatment at the doses currently recommended, the IGF1/IGFBP3 molar ratio remained within the normal range for age and sex, even during puberty. We also found that, in children treated with GH, significant increases in serum IGF1 levels were sometimes associated with IGF1/IGFBP3 molar ratios within the normal range for age and pubertal stage. This suggests that, even in cases of high IGF1 concentration, the action of this molecule is counteracted by an adaptation of IGFBP3 levels, decreasing IGF1 bioavailability.

Study Limitations
This study included a small number of subjects and our results therefore require confirmation in a larger cohort.

Conclusion
We consider IGF1/IGFBP3 molar ratio to be a useful additional parameter for assessments of treatment safety and for the adjustment of rGH treatment. The goal would be to maintain this ratio within the normal reference range for age and pubertal stage.

Ethics: All procedures involving human participants in this study were performed in accordance with the French national rules for ethics and confidentiality, in accordance with current legislation for retrospective non-interventional studies of cohort files.

Informed consent: All clinical data and biological analyses were performed as part of routine follow-up, without the collection of additional samples. Under French law, no specific consent for this anonymous study was therefore required. Concerning the ratio reference values of the children controls, the families gave their consent to use the remains of the samples of the explorations.


Conflict of interest: The authors declare that they have no conflicts of interest directly relating to this study. However, YLB has had congress registration, transport, accommodation paid by Sandoz, Ipsen and has received honoraria as a lecturer and speaker from Sandoz, Ipsen, and Novo Nordisk.

Financial Disclosure: No financial disclosure.

References
Table 1a. Clinical and hormonal characteristics of the children

<table>
<thead>
<tr>
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<th>SGA Group</th>
<th>GHD Group</th>
<th>PWS Group</th>
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<tbody>
<tr>
<td><strong>Clinical data</strong></td>
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<tr>
<td>Number of children:</td>
<td>20</td>
<td>61</td>
<td>11</td>
</tr>
<tr>
<td>Age (years)</td>
<td>12.27 ± 3.50 (5.9 – 18.9)</td>
<td>12.02 ± 4.09 (1.4 – 18.9)</td>
<td>10.88 ± 5.23 (1.4 – 16.4)</td>
</tr>
<tr>
<td>Boys/girls</td>
<td>15/5</td>
<td>41/20</td>
<td>6/5</td>
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<tr>
<td>Weight (SDS)</td>
<td>-0.71 ± 2.52</td>
<td>0.83 ± 2.46</td>
<td>0.83 ± 2.46</td>
</tr>
<tr>
<td>Height (SDS)</td>
<td>1.46 ± 0.87</td>
<td>-0.97 ± 1.16</td>
<td>-0.76 ± 1.53</td>
</tr>
<tr>
<td>BMI (SDS)</td>
<td>0.57 ± 2.44</td>
<td>0.58 ± 1.88</td>
<td>1.51 ± 1.86</td>
</tr>
<tr>
<td><strong>Biological data</strong></td>
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<tr>
<td>IGF1/IGFBP3 ratio before rGH</td>
<td>0.14 ± 0.04</td>
<td>0.07 ± 0.01</td>
<td>0.12 ± 0.02</td>
</tr>
<tr>
<td>IGF1/IGFBP3 ratio after rGH</td>
<td>0.19 ± 0.07</td>
<td>0.20 ± 0.08</td>
<td>0.19 ± 0.09</td>
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<tr>
<td>Samples, <em>n</em></td>
<td>23</td>
<td>18</td>
<td>15</td>
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<tr>
<td><strong>Puberty stage</strong></td>
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<td>P1</td>
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<tr>
<td>P2-P5</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td><strong>Age (years)</strong></td>
<td>0 - 4</td>
<td>≥ 4 - 8</td>
<td>≥ 8 - 10</td>
</tr>
<tr>
<td>Mean IGF1/IGFBP3 molar ratio</td>
<td>0.10</td>
<td>0.12</td>
<td>0.14</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>0.05</td>
<td>0.03</td>
<td>0.03</td>
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<tr>
<td>Range</td>
<td>0.03 – 0.24</td>
<td>0.08 – 0.18</td>
<td>0.10 – 0.21</td>
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</table>
Table 2. Clinical and metabolic characteristics of children on rGH with molar ratios higher or lower than the normal range

<table>
<thead>
<tr>
<th></th>
<th>Low Ratio (n=41)</th>
<th>Normal Ratio (n=33)</th>
<th>High Ratio (n=18)</th>
<th>ANOVA</th>
<th>Post hoc ANOVA</th>
<th>P Values</th>
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<tbody>
<tr>
<td></td>
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<td></td>
<td>F-Value</td>
<td>P Value</td>
<td>Low vs. Normal</td>
</tr>
<tr>
<td>Ratio (Post rGH)</td>
<td>0.12 ± 0.02</td>
<td>0.24 ± 0.03</td>
<td>0.32 ± 0.02</td>
<td>467.56</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Age (years)</td>
<td>9.92 ± 4.77</td>
<td>12.61 ± 2.85</td>
<td>14.19 ± 2.01</td>
<td>10.00</td>
<td>0.0001</td>
<td>0.0051</td>
</tr>
<tr>
<td>BMI (SDS)</td>
<td>0.41 ± 1.63</td>
<td>0.31 ± 1.01</td>
<td>0.18 ± 1.30</td>
<td>0.16</td>
<td>0.84</td>
<td>0.7661</td>
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<tr>
<td>Height (SDS)</td>
<td>-0.85 ± 1.10</td>
<td>-0.95 ± 1.41</td>
<td>-1.38 ± 0.99</td>
<td>1.19</td>
<td>0.30</td>
<td>0.7228</td>
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<tr>
<td>Dose of rGH (µg/kg/ day)</td>
<td>24.99 ± 9.46</td>
<td>28.33 ± 9.82</td>
<td>39.67 ± 17.20</td>
<td>8.82</td>
<td>0.0003</td>
<td>0.1989</td>
</tr>
<tr>
<td>Insulin (mIU/l)</td>
<td>5.47 ± 3.30</td>
<td>5.85 ± 3.15</td>
<td>9.77 ± 4.17</td>
<td>7.25</td>
<td>0.0017</td>
<td>0.7450</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>5.33 ± 0.36</td>
<td>5.07 ± 0.28</td>
<td>5.40 ± 0.34</td>
<td>1.87</td>
<td>0.17</td>
<td>0.0976</td>
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<tr>
<td>Fasting glycemia (mmol/l)</td>
<td>4.72 ± 1.30</td>
<td>4.77 ± 0.25</td>
<td>4.80 ± 0.29</td>
<td>0.03</td>
<td>0.96</td>
<td>0.8974</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>4.00 ± 0.64</td>
<td>4.18 ± 1.13</td>
<td>3.60 ± 0.59</td>
<td>1.57</td>
<td>0.22</td>
<td>0.6327</td>
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<tr>
<td>HDL-cholesterol (mmol/l)</td>
<td>1.63 ± 0.38</td>
<td>1.66 ± 0.56</td>
<td>1.46 ± 0.17</td>
<td>0.65</td>
<td>0.52</td>
<td>0.9131</td>
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<tr>
<td><strong>LDL-cholesterol (mmol/l)</strong></td>
<td>1.96 ± 0.49</td>
<td>2.06 ± 0.75</td>
<td>1.9 ± 0.47</td>
<td>0.19</td>
<td>0.82</td>
<td>0.6973</td>
</tr>
<tr>
<td><strong>Triglycerides (mmol/l)</strong></td>
<td>0.61 ± 0.29</td>
<td>0.73 ± 0.38</td>
<td>0.77 ± 0.30</td>
<td>0.92</td>
<td>0.40</td>
<td>0.3828</td>
</tr>
</tbody>
</table>
Figure 1. Distribution of IGF1/IGFBP3 ratio in the three groups before (a) and after (b) rGH treatment. SGA: small for gestational age; GHD: growth hormone deficiency; PWS Prader Willi Syndrome. Rectangles represent values between +1 and -1 SDS and bars represent SDS values.
Figure 2. Distribution of IGF1 concentration (a) and IGF1/IGFBP3 molar ratio (b), in SGA children during GH treatment. The IGF1 values have been distributed according to the SDS intervals established by Bidlingmaier et al (10). In the group SGA group, 2 children had IGF1 concentrations > +2SDS, but IGF1/IGFBP3 molar ratios in the normal range (arrows).
Figure 3. Distribution of IGF1 (a) and IGF1/IGFBP3 molar ratio (b), in children with GHD during GH treatment. In the group GHD group, 8 children had IGF1 concentrations > +2SDS (circled cases), but IGF1/IGFBP3 molar ratios in the reference range. Conversely, one five-year-old child had an IGF1 concentrations in the reference range but a very high molar ratio due to a very low IGFBP3 concentration (<-2SDS; arrow).
Figure 4. Distribution of IGF1 (a) and IGF1/IGFBP3 molar ratio (b), in children with PWS during GH treatment.
In the PWS group, 3 children had discrepancies between serum IGF1 levels (expressed in SDS) and IGF1/IGFBP3 molar ratio. 1st case (arrow 1): 1.4-year-old child with PWS and an IGF1 concentration in the normal range (expressed in SDS) but a low molar ratio (due to very high IGFBP3 concentration > +2 SDS). 2nd case (arrow 2): 5.4-year-old child with PWS and an IGF1 concentration in the normal range (expressed in SDS) but a high IGF1/IGFBP3 molar ratio (due to low IGFBP3 concentration). 3rd case (arrow 3): 11.4-year-old child with a high IGF1 concentration (expressed in SDS) and an IGF1/IGFBP3 molar ratio in the reference range (IGFBP3 concentration towards the upper end of the reference range).