Growth Hormone:
…from test tube to therapy…

Gérald Theintz
Professeur Honoraire
Faculté de Biologie & Médecine
UNIL
Expert scientifique Médisupport

Journée ARL - 29.03.12 - Yverdon

1. Growth hormone ≠ growth only: beneficial effects
2. Mode of action and problems of dosage
3. Clinical factors altering GH values and difficulty in interpreting the results
4. Conclusions
Beneficial Effects of GH

- **Growth**
  - multiplication and division of cartilage chondrocytes
  - production of IGF-1
- **Protein metabolism**
  - increase of protein synthesis and amino acid uptake
  - decrease of protein oxidation
- **Fat metabolism**
  - Enhancement of fat utilization (promotes lipolysis)
- **Carbohydrate metabolism**
  - promotion of gluconeogenesis in the liver
  - reduction of glucose liver uptake
- **Additional benefits**
  - Increase of calcium retention and bone mass
  - Increase of muscle mass
In the past, critical studies on diagnosis and therapy of GH related diseases have reported GH levels measured by competitive assays based on polyclonal antibodies.

The outcome of these studies still influences present guidelines and clinical practice although these methods are not used anymore.

External quality assessment schemes document large method or laboratory dependent discrepancies.

Variability for a single sample measured by different methods exceeds 100% which makes it impossible to compare GH assay results when different assays and laboratories are used.

In addition, studies illustrate that assay discrepancies affect the clinical interpretation of the results.

---

**Measuring GH: a challenge (1)**

- In the past, critical studies on diagnosis and therapy of GH related diseases have reported GH levels measured by competitive assays based on polyclonal antibodies.
- The outcome of these studies still influences present guidelines and clinical practice although these methods are not used anymore.
- External quality assessment schemes document large method or laboratory dependent discrepancies.
- Variability for a single sample measured by different methods exceeds 100% which makes it impossible to compare GH assay results when different assays and laboratories are used.
- In addition, studies illustrate that assay discrepancies affect the clinical interpretation of the results.

---

**GH isoforms in circulation**

<table>
<thead>
<tr>
<th>GH Isoform</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>22 kDa monomer total</td>
<td>48</td>
</tr>
<tr>
<td>20 kDa monomer total</td>
<td>9</td>
</tr>
<tr>
<td>Acidic GH (desamido- &amp; acyl-GH)</td>
<td>5</td>
</tr>
<tr>
<td>22 kDa non-covalent dimers</td>
<td>14</td>
</tr>
<tr>
<td>22 kDa disulphide dimers</td>
<td>6</td>
</tr>
<tr>
<td>20 kDa non-covalent dimers</td>
<td>5</td>
</tr>
<tr>
<td>22 kDa non-covalent oligomer</td>
<td>7</td>
</tr>
<tr>
<td>22 kDa disulphide oligomer</td>
<td>3</td>
</tr>
<tr>
<td>Others</td>
<td>3</td>
</tr>
</tbody>
</table>

Bildlingmaier M. 2008
Endocrine Research Laboratories, Ludwig-Maximilians University, Munich
Commonly used commercial assays for GH

<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>Name</th>
<th>Calibration</th>
<th>Isoform specif.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Siemens</td>
<td>Immulite 2000</td>
<td>98/574-80/505</td>
<td>Not provided</td>
</tr>
<tr>
<td>DiaSorin</td>
<td>Liaison hGH</td>
<td>98/574</td>
<td>Not provided</td>
</tr>
<tr>
<td>Beckmann-Coulter</td>
<td>Access ultra s hGH</td>
<td>98/574-80/505</td>
<td>20 kD &lt; 4%</td>
</tr>
<tr>
<td>IDS</td>
<td>iSys hGH</td>
<td>98/574</td>
<td>20 kD &lt; 2%</td>
</tr>
<tr>
<td>Perkin-Elmer (Wallac)</td>
<td>DELFIA hGH</td>
<td>80/505</td>
<td>22 kD specific</td>
</tr>
<tr>
<td>BioSource</td>
<td>hGH IRMA</td>
<td>98/574</td>
<td>Not provided</td>
</tr>
<tr>
<td>Adaltis</td>
<td>hGH Bridge</td>
<td>80/505-88/624</td>
<td>Not provided</td>
</tr>
<tr>
<td>Cisbio</td>
<td>hGH-RIACT</td>
<td>98/574</td>
<td>20 kD &lt; 5%</td>
</tr>
<tr>
<td>DSL</td>
<td>Active hGH Elisa</td>
<td>80/505-88/624</td>
<td>Not provided</td>
</tr>
</tbody>
</table>

Biddingmaier M et al, Growth Horm & IGF Res 2010; 20: 19

Measuring GH: a challenge (2)

- GH in serum samples remains stable for 24 h at room temperature.
- It is a matter of debate whether a more specific or a more permissive recognition of GH isoforms is preferable from a clinical point of view.
- It has convincingly been shown that different isoforms are biologically active: one is therefore missing information with assays that measure only one isoform.
- The "translation" of GH results from one assay to another is also hampered by the role of GHBPs in the assays.
- Some important journals specialized in the field have decided to only publish data expressed in mass units of the IRP 98/574.
- However, for a given reference preparation (i.e. 98/574), the matrix of the assay specific calibrators varies between assays from different manufacturers (calibrate the calibrator !)
Urinary Growth Hormone

- Same methodological problems (isoforms)
- Very low amounts (< 0.01%) → 1'000x more sensitive methods
- Physicochemical characteristics more variable than in the blood

Take home message

U-GH determination remains very controversial in the diagnosis of GH deficiency, even when measured in 24 hr collections and corrected for creatinine.


• In basal (random) conditions
  - What do we expect to obtain:
    - High vs low diagnostic levels?
  - Effect of feeding (acute) / overfeeding?
  - GH is a stress hormone
  - Can a so-called normal range be applied?
• In serial sampling (overnight)
• IGF-1 ± GH response to provocative testing
GH Response to Physical Exercise

Ex I: 4 min [90-100% VO₂ max]
Ex II: 30 min [60-70% VO₂ max]

Plasma GH (ng/ml x 10⁻³)

Ex I: 4 min [90-100% VO₂ max]
Ex II: 30 min [60-70% VO₂ max]

n = 9
n = 11

Integrated responses of GH, ACTH and Cortisol above basal after IV insulin in 4 overtrained athletes

Adapted from Barron JL et al, JCEM 1985; 60: 803
Effect of weight on GH response to GHRH

Take home message

- Random measurements of plasma growth hormone are of poor value for diagnostic purposes in the majority of cases due to various uncontrollable parameters.

- Random GH single measurements are therefore not recommended as a first line determination.
• In basal (random) conditions
  - problem: GH is a stress hormone
  - effect of feeding (acute) / overfeeding?
  - what do we expect to obtain:
    high vs low diagnostic levels?
  - can we apply a so-called normal range?
• In serial sampling (overnight)
  • IGF-1 ± GH response to provocative testing

Circadian GH Secretory Pattern

<table>
<thead>
<tr>
<th>Time clock</th>
<th>Plasma GH (μg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>00:00</td>
<td>0</td>
</tr>
<tr>
<td>02:00</td>
<td>4</td>
</tr>
<tr>
<td>04:00</td>
<td>8</td>
</tr>
<tr>
<td>06:00</td>
<td>12</td>
</tr>
<tr>
<td>08:00</td>
<td>16</td>
</tr>
<tr>
<td>10:00</td>
<td>20</td>
</tr>
<tr>
<td>12:00</td>
<td>24</td>
</tr>
<tr>
<td>14:00</td>
<td>28</td>
</tr>
<tr>
<td>16:00</td>
<td>32</td>
</tr>
<tr>
<td>18:00</td>
<td>36</td>
</tr>
<tr>
<td>20:00</td>
<td>40</td>
</tr>
<tr>
<td>22:00</td>
<td>44</td>
</tr>
<tr>
<td>00:00 (next day)</td>
<td>48</td>
</tr>
</tbody>
</table>

Sleep study

Meals

23 y
Daytime Pulsatile GH Secretion during Childhood and Adolescence

Miller JD et al, JCEM 1982; 55, 989

Take home message

- Overnight (sleep) serial blood sampling represents a good physiological approach to diagnose GH deficiency or overproduction.
- However, it is reserved to highly specialized centres. It has a very poor cost effectiveness.
- It is therefore very rarely used taking into account that diagnosis can be established for the majority of cases using IGF-1 measurement ± GH provocative testing.
• In basal (random) conditions
  - problem: GH is a stress hormone
  - effect of feeding (acute) / overfeeding ?
  - what do we expect to obtain:
    high vs low diagnostic levels ?
  - can we apply a so-called normal range ?
• In serial sampling (overnight)
• IGF-1 ± GH response to provocative testing

IGF-1 : a tool to use with caution for the diagnosis of GH related diseases
Insulin Growth Factors as Diagnostic Tools in GH Deficiency: The KIGS Experience

GH deficient children (n = 187)

NON-GH deficient children (n = 205)

IGF1 Response to Exercise or Fasting

6 trained males aged 20-31 yrs

Smith A.T. et al, Metabolism 1987:36, 533

IGF-1

- Reference values
- Units (nmol/l vs μg/l)

Blum, 1990

Elmlinger, 2004

+2 DS

-2 DS

M

0 100 200 300 400 500

IGF-1 (μg/ml)

0 4 8 12 16 20

Age

0 200 400 600 800 1000

+2 DS

-2 DS

M

0 4 8 12 16 20

Age
Limited applicability of international consensus criteria to local practice

Aliquots of 1 sample sent to 23 labs

- 6 different assays (23 labs)
- 15 different ref. ranges (23 labs)
- ref. ranges from the kit (15 labs)
- Inhouse ref. ranges (7 labs)
- 2-fold variation in upper limit


GH stimulation / suppression tests

- Insulin induced hypoglycaemia
- Glucagon (± propanolol)
- Arginine perfusion
- Clonidine (α-adrenergic stimulant)
- L-dopa (± propanolol)
- GHRH
- Calibrated physical exercise (ergometer)
- [IGF-1 generation test]
  
  Glucose load test
Arginine-Insulin combined test
Supposed to be the « gold » standard

Growth Hormone (μg/l)

Arginine perfusion
Insulin IV

GH normal response
GH partial Deficiency
GH complete Deficiency

Time (minutes)

Glycaemia (mmol/l)

Cortisol stimulation Test
± propanolol premedication

Cortisol
Growth hormone
Glycaemia

Time (minutes)
Immulite 2000 vs Roche Elecsys assays for plasma GH during arginine stimulation testing

Take home message

• In children with a suspicion of growth failure, IGF1 and IGFBP3 should be measured first and correlated to growth velocity, weight, bone age and pubertal stage.

• In “grey” situations, these measurements can be repeated (2-3 months later) and, eventually, a GH stimulation test performed.

• The diagnosis of GH deficiency is established when 2 consecutive tests (using different stimuli) yield positive results. There is no general consensus on the lower limit of normal.

• Subjects should be retested after therapy completion.
1. Growth hormone ≠ growth only and its beneficial effects
2. Mode of action and problems of dosage
3. Clinical factors altering GH values and difficulty in interpreting the results
4. Conclusions

Conclusions (1)

- GH acts via a mainly nocturnal pulsatile secretion through complex secretory and control mechanisms.
- Most of its effects are mediated by IGF1 and IGFBP3 which are main actors in the growth process.
- GH is produced and secreted into the circulation in several biologically active isoforms.
- Commonly used GH kits yield discordant results even with the more recent monoclonal antibodies. It also applies to kits measuring IGF1.
Conclusions (2)

• To-day’s clinical practice remains essentially based on “old” methodology. Establishing new reference data requires: a) considerable resources; b) cohorts of normal subjects, which may be impossible to recruit due to ethical reasons.

• In addition, labs working in a high competitive economic environment are very likely unable to produce such references values for any new GH assay introduced.

• “The devil is in the detail”… An acceptable compromise is to: a) use kits yielding results which are familiar to endocrinologists; b) limit as much as possible the variety of kits used in a regional area.