Beyond the Basics of Endocrine Stimulation Testing
Las Vegas, NV

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Conflict of Interest Disclosure

- Advisory Board Pfizer
- Advisory Board GenenTech
- Speaker Bureau for Novo Nordisk

There is no COI of the above mentioned with the presented material.
OBJECTIVES

- The purpose of the most common provocative stimulation testing
- The most common agents used in the different types of stimulation testing
- Adverse effects of the agents
- Timing of medications and the role it plays and other factors that may impact results
- Interpretation of the common tests

Growth Hormone Stimulation

Traditional means for documenting growth hormone deficiency.
Measuring serial samples of the growth hormone after administration of growth hormone secretagogues to acutely stimulate pituitary growth hormone production
Diagnosis is based on demonstration of an inadequate rise of serum growth hormone.

GH Stimulation Testing Agents

- Arginine
- Clonidine
- Insulin
- Glucagon
- L-Dopa
GH Stimulation Testing Agents, Dosage and Adverse Effects

- Arginine:
  - Dose: 0.5 g/kg IV of 10% solution over 30 minutes. Maximum 20 grams.
  - Samples at 0, (15), 30, (45) and 60 minutes
  - Peak Time of GH: 30-60 min.
  - Side Effects: late hypoglycemia, blood in urine.

- Clonidine
  - Dose: 5 ug/kg to a maximum of 250 ug or 0.1 to 0.15 mg/m2 (depending on protocol)
  - Samples at 0, 30, 60 and 90 minutes
  - Peak Time of GH: 60 minutes (90 minute sampling will usually be 30% lower).
  - Side Effects: Drowsiness (in young children may last several hours), Drowsiness may cause hypoglycemia due to extended fasting. Central postural hypotension, nausea. Central respiratory depression with overdose.
  - Monitor blood pressure at 0,30, 60 and 90 minutes

- Glucagon
  - Dose: 0.03 mg/kg to a maximum of 1 mg IM/SC
  - Samples at 0, 1, 2, 2.5 and 3 hours.
  - Peak Time of GH: 2-3 hours
  - Side Effects: Late hypoglycemia. Nausea and vomiting in young children
  - Other: Best choice in young children and infants. Good substitute for insulin tolerance test that could be risky for newborn and small children because it induces GH secretion by stimulating endogenous insulin secretion to compensate for elevated glucose levels.
GH Stimulation Testing
Agents, Dosage and Adverse Effects

• Insulin
  – Dose: 0.05 -0.1 U/kg
  – Samples at 0, 15, 30, 45 ,60, (75), 90 and (120) minutes
  – Peak Time of GH: 30-60 minutes
  – Side Effects: Severe hypoglycemia (requires close supervision). Not recommended in infants and small children or those with hypopituitarism with a risk of adrenal insufficiency.
  – Considered the “gold standard” but is risky
  – Cortisol reserve can be assessed at the same time.

• L-Dopa (not available in US. Must be compounded)
  – Dose: 125mg for <15 kg; 250 mg for 15-35kg; 500 mg over 35 kg.
  – Samples at 0, 60 and 90 minutes
  – Side Effects: Nausea and vomiting especially in toddlers. Headache

GH Stimulation Testing
Additional Considerations

Sex Steroid Priming may be appropriate in the immediate period of prior to the onset of puberty since during this period they secrete less GH then pubertal subjects and may have significantly reduced growth hormone values that are not representative of true growth hormone deficiency.
GH Stimulation Testing
Additional Considerations

- Sex Steroid Priming Protocols
  - Premarin 5 mg po the night before and the morning of the test (or)
  - Ethinyl Estradiol 50-100 ug/d for 3 consecutive days before testing (or)
  - Depot testosterone 100 mg 3 days before testing
  OR
  - Ethynil-Estradiol: Can be used on both males and females (0.02 mg for less than 50 lbs; 0.05 mg for over 50 lbs). Given 18 hours, 12 hours and 1 hour before test
  - Depot testosterone: 100 mg 7-10 days before test

GH Stimulation Testing
Preparation

- Time of Day for Test
  - Should be performed while fasting
  - There may not be any physiological basis for food restriction. However, normal and expected values are based on testing in morning.

Interpreting GH Stimulation Tests

- Generally recognized criteria for GH deficiency are responses of less than 10 ng/mL to different pharmacological agents.
- Tests are non physiologic in that children who have hypothalamic dysregulation due to cranial irradiation may mount a normal pituitary growth hormone response to stimulation but under normal basal conditions have subnormal spontaneous GH secretion.
- Many conflicting reports of the validity of this test for the basis of diagnosing GHD
GnRH/Lupron Stimulation

Useful for evaluating the role of pituitary function in children with premature or delayed puberty. Responses can be used to distinguish between central nervous system dysfunction such as hamartoma or craniopharyngioma from peripheral dysfunction (McCune-Albright, testotoxicosis, ovarian follicular cysts) in children with premature puberty. In delayed puberty it helps with distinguishing between peripheral dysfunction (Turner’s, Klinefelter) and Kallman, Prader Willi and constitutional delay.

GnRH (Lupron) Stimulation Testing Agents, Dosage and Adverse Effects

- Leuprolide Acetate (Facteral not available)
  - Dose: 20 mcg/kg to a maximum of 1500 mcg SC
  - Samples at 0, 30, 60, 90, 120 and (180 minutes) for LH, FSH.
  - Sex steroid of interest at 2 hours and (24 hrs)
  - Peak: 3-4 hours after administration
  - Side Effects:
    - Hypersensitivity. Rash, urticaria, and photosensitivity reactions have been reported. Anaphylactoid or asthmatic process reported rarely.
    - Pituitary apoplexy, a clinical syndrome resulting from infarction of the pituitary gland, reported rarely. Most cases occur within 2 weeks of the first dose, sometimes within the first hour. If manifestations (e.g., sudden headache, vomiting, visual changes, ophthalmoplegia, altered mental status, sometimes cardiovascular collapse) occur, immediate medical attention required.

Interpreting GNRH (Lupron) Stimulation Tests

- LH levels increase into a pubertal range in the presence of puberty.
- FSH levels may not change discernibly.
- Prepubertal children (especially girls) have relatively greater FSH basal and stimulated levels then LH.
Interpreting GNRH (Lupron) Stimulation Tests

- Central Precocious Puberty.
  - In early or normal puberty –
    - Hypothalamic Gnrh is produced in a pubertal pattern that primes the pituitary gonadotrophs.
    - So that following the administration of a one time pharmacological dose of Gnrh there is an abundant release of Lh as typically occurs in teenagers.

GnRH stimulation tests results differentiating responses between girls with precocious puberty and prepuberty

(Lee and Houk, Pediatric Endocrinology. 5th edition, 2007)

Interpreting Gnrh (Lupron) Stimulation Tests for CPP

Ibanez et al (86)and Garibaldi et al (87)
LH above 9.7 IU/L or 9.7 miU/mL
Testosterone level above 3.5 nmol/L (100 ng/dL) in males
Estradiol level above 2.49 pmol/L (9 pg/mL)
Pubertal Response in CPP

<table>
<thead>
<tr>
<th>Time</th>
<th>FSH (mIU/mL)</th>
<th>LH (mIU/mL)</th>
<th>T (ng/dL)</th>
</tr>
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<tbody>
<tr>
<td>Baseline</td>
<td>1.6</td>
<td>1.1</td>
<td>+1</td>
</tr>
<tr>
<td>1 Hour</td>
<td>3.1</td>
<td>15</td>
<td>593</td>
</tr>
<tr>
<td>2 Hour</td>
<td>4.1</td>
<td>11</td>
<td>826</td>
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</table>

Interpreting GNRH (Lupron) Stimulation Tests

• Delayed Puberty due to gonadal failure
  – If gonadotropin levels mildly elevated then GnRH stimulation may unmask excessive response of LH and FSH documenting primary gonadal failure.
  – If gonadotropin levels are clearly elevated then GnRH stimulation testing is unnecessary.
  – Limited usefulness for differentiating gonadotropin deficiency from constitutional delay.
  – Should not be performed until bone age is at least 10-11 years for girls and 12-13 years for boys.

ACTH Stimulation

Most reliable tool for diagnosing 21 hydroxylase deficiency in patients with CAH.
The widest use of ACTH stimulation test is for the purpose of diagnosing CAH.
Stimulating the adrenal with ACTH increases steroidogenesis resulting in accumulation of steroids proximal to the disordered enzyme.
Also, used in diagnosing Addison disease.
ACTH Stimulation Testing
Agents, Dosage and Adverse Effects

• Cortrosyn (synthetic ACTH)
  – Medications: Should not be receiving medications that interfere with ACTH secretion, especially high dose glucocorticoids or other steroids.
    • High dose steroids must be discontinued for at least one week to permit restoration of reserve.
    • If on chronic steroid treatment the withdrawal process may take several months.
    • Not done in first 24 hours of life (ambiguous results)

ACTH Stimulation Testing
Agents, Dosage and Adverse Effects

• Dose: Single IV bolus of 0.25 mg administered after baseline sample drawn. (Dose is used for all ages including newborns and premature infants. However, some resources recommend 15 ug/kg under age 2.)
  – Samples at 0 and 60 minutes
    • Cortisol
    • 17 OHP
    • Progesterone
    • 17 hydroxypregnenolone
    • CAH 6
  – Side Effects:
    • Cardiovascular: Bradycardia, hypertension, peripheral edema, tachycardia
    • Dermatologic: Rash
    • Local: Wheals with redness at the injection site
    • Miscellaneous: Anaphylaxis, hypersensitivity reaction

Interpreting ACTH Stimulation Tests for 21 hydroxylase deficiency if not using CAH 6 Panel

Sum of the increase in progesterone and 17 hydroxyprogesterone concentrations is divided by the time between samples.

Heterogenous individuals for CAH have responses between 9-30 ng/dL/minutes

Responses below 7 ng/dL/minutes are typical of normal subjects.
Interpreting ACTH Stimulation Tests
3 B-hydroxysteroid dehydrogenase deficiency if not using CAH 6

Ratio of 17 hydroxypregnenolone to 17 hydroxyprogesterone levels
Normal individuals have a ratio of less then 10 and higher ratios are considered diagnostic.

CAH 6 Panel for ACTH Stimulation

<table>
<thead>
<tr>
<th>Test</th>
<th>Baseline</th>
<th>Stimulated</th>
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<tbody>
<tr>
<td>17-OHP</td>
<td>11.8</td>
<td>13.6</td>
</tr>
<tr>
<td>4-androstenedione</td>
<td>58</td>
<td>136</td>
</tr>
<tr>
<td>DOC</td>
<td>16</td>
<td>43</td>
</tr>
<tr>
<td>Testosterone</td>
<td>&lt;10</td>
<td>53</td>
</tr>
<tr>
<td>Progesterone</td>
<td>122</td>
<td>54</td>
</tr>
<tr>
<td>Cortisol</td>
<td>72</td>
<td>24</td>
</tr>
<tr>
<td>17-OH-progesterone</td>
<td>258</td>
<td>103</td>
</tr>
<tr>
<td>Cortisol</td>
<td>240</td>
<td>98</td>
</tr>
<tr>
<td>DHEA</td>
<td>458</td>
<td>113</td>
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Interpretation with CAH 6 panel
- Impaired activity of P450c21 (21 hydroxylase) should lead to the accumulation of progesterone and 17 OHP.
- Progesterone does not accumulate in appreciable quantities because it converts to 17 OHP.
- Comparing the patients basal to ACTH stimulated values of 17 OHP against a large number of well studied patients usually permits discrimination.
- Tables provided with the normal values of basal and stimulated with the diagnostic criteria.
Interpreting Standard ACTH Stimulation Tests for Addisons

- Cortisol levels should exceed 16 ug/dL (some references list higher values).
- The stimulation test helps to differentiate between primary and secondary causes of adrenal insufficiency.
- In secondary (hypopituitarism) some steroidogenic capacity is present. Therefore, some cortisol is produced in response to ACTH stimulation.
- In primary (Addisons) the cortisol secretion is negligible.

Low Dose ACTH Stimulation

Used to evaluate the integrity of the hypothalamic – pituitary – adrenal axis and adrenal reserve. Low dose ACTH test has more sensitivity in detecting subtle states of adrenal insufficiency and provides a more physiological concentration of ACTH. Useful in testing individuals who may have mild insufficiency due to inhaled steroids.

ACTH Stimulation Testing Agents, Dosage and Adverse Effects

- Cortrosyn
  - Dose: Single 1 mcg IV bolus
  - Samples at 0, 30 and (60 minutes)
- Side Effects
  - Cardiovascular: Bradycardia, hypertension, peripheral edema, tachycardia
  - Dermatologic: Rash
  - Local: Wheals with redness at the injection site
  - Miscellaneous: Anaphylaxis, hypersensitivity reaction
Interpreting Low Dose ACTH Stimulation Tests for Adrenal Insufficiency

- Serum cortisol level of at least 16 ug/dL
- Some references suggest greater than 20 ug/dL.

<table>
<thead>
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<th>Cortisol (ug/dL)</th>
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</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>25</td>
<td>5.8</td>
</tr>
<tr>
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</tr>
<tr>
<td>60 minutes</td>
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Adrenal Insufficiency

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<th>Cortisol (ug/dL)</th>
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<td>60 minutes</td>
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Partial Deficiency

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<td>51</td>
<td>5.1</td>
</tr>
<tr>
<td>30 minutes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>60 minutes</td>
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<td>60 minutes</td>
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Conclusion

- Hormonal dynamic testing is a useful tool in diagnosing a variety of endocrine disorders.
- Careful consideration must be given to the administration of the test to provide the most reliable results.
- Understanding how tests are interpreted aids in providing families with most accurate information about simulation tests.

Children’s Hospital & Medical Center

- 145 beds
- Nebraska’s only full-service pediatric specialty health care center
- Only Level IV Regional NICU in Nebraska
- ACS-verified Level II Pediatric Trauma Center
- 370,000 patient visits annually
- 59,400 specialty outpatient visits