ENDOCRINOLOGY

Syllabus

labcorp

Endocrinology Syllabus

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Labcorp is proud to offer a comprehensive test portfolio for endocrinology testing. We recognize the unique needs of healthcare providers who are diagnosing and managing endocrine disorders. In addition to our routine lab offerings, we also offer many highly sensitive and specific test options using alternative methods.

About Labcorp's Endocrinology Center of Excellence Services

Labcorp's Endocrinology Center of Excellence (formerly branded Endocrine Sciences), which is located at Labcorp's Esoterix, Inc. laboratory (see information below), is a research-quality reference laboratory specializing in highly specific and sensitive endocrine testing services. Our laboratory provides a unique service oriented to the distinct requirements of an endocrinology practice. Labcorp is an industry leader in developing new, clinically relevant assays and establishing defined reference intervals for pediatric and adult patient populations. All laboratory testing is conducted by highly qualified professionals, specifically trained to provide knowledgeable, responsive attention to client needs. Labcorp's accessible scientists and physicians are available to provide technical consultation and assist with clinical evaluations. Our goal is to employ technology to provide useful clinical information—not just laboratory data—to affect patient care and the delivery of cost-effective medical treatment in a positive manner. We are your source for endocrine testing and expertise.

Capabilities

- Extensive menu of laboratory assays useful in the following areas:
  - Diabetes
  - Bone metabolism
  - Thyroid function
  - Growth
  - Hypertension
  - Adrenal function
  - Sexual development and reproduction
- Technical and clinical consultation regarding assay methods
- Comprehensive age-related reference intervals for accurate interpretations
- Strong pediatric focus
- Exceptionally sensitive and specific assays

Lab Location and Hours of Operation

Address: Esoterix, Inc, 4301 Lost Hills Road, Calabasas Hills, Calif 91301
Phone: 800-444-9111, option 1
Fax: 512-225-1253
Monday – Friday 7:00 am – 8:00 pm CST
Saturday 8:00 am – 5:00 pm CST

Lab License Numbers (Esoterix, Inc)

CLIA	05D0663070
CAP	2298301
Medicare	05D0663070
CA	CLF2343
FL	L800004459
MD	589
NY	3072
PA	021822A
RI	LCO00265

Special Specimen Collection Procedures for Endocrinology Tests

The accuracy of endocrine testing depends on the quality of the specimen submitted. Endocrine specimens must be properly collected, labeled, stored, packaged, and transported. Phlebotomists and specimen managers must carefully adhere to all time limits. Following these instructions properly will assure specimen integrity and contribute to valid, consistent endocrine results.

Prior to collecting the specimen, review the specimen requirements listed in the general menu section. Note the proper specimen type to be collected, amount of specimen necessary for testing, and special storage and shipping instructions. The specimen container must be properly identified with the patient's full name, identification number, date and time of collection and specimen type.

A properly completed test request form (TRF) must accompany each specimen. It is important that the age and/or birthdate of the patient be indicated so that age-adjusted normal values can be provided.

CLIA regulations further require that we receive physician signatures on additional tests ordered or test changes not accompanying the original TRF.

A more complete test menu, including specimen requirements and reference intervals, can be found in our online test menu at Labcorp.com.

Salivary Cortisol

Saliva should be collected at the precise time(s) prescribed by the physician. Saliva specimens can be collected manually by spitting into a clean sample vial, however the preferred method is to collect with the aid of a Salivette. The Salivette is a device made specifically for the purpose of collecting salivary cortisol specimens. If not using a Salivette, be sure to collect at least 1 mL of saliva. Regardless of collection technique, please observe the following guidelines:

1. Instruct the patient to collect saliva at the times prescribed by the physician.
2. No food or fluid should be consumed 30 minutes prior to collection.
3. The patient should not apply creams or lotions that contain steroids or use steroid inhalers for 24 hours prior to collection. (This is to avoid contamination of the Salivette or collection vial.)
4. The patient should avoid activities that can cause your gums to bleed. This would include brushing or flossing.
5. The patient should not collect and submit a sample if their gums or the inside of their mouth is bleeding.
6. The sample vial or Salivette should be labelled with the patient's name, physician's name, and the time and date of collection.
7. Instruct the patient to return the specimen to the physician office or Labcorp patient service center within one day of collection.
8. If necessary, salivation can be stimulated with sugar-free KoolAid® powder.

Urine, 24-Hour Collection

Most urine assays require a 24-hour specimen because of diurnal variations in excretion of many hormones. Patients should receive explicit instructions for obtaining a complete 24-hour urine sample. Start the 24-hour period at exactly 7:00 am the first morning by voiding. Discard this first voiding since the urine was formed prior to the collection period. Collect all subsequent voidings until 7:00 am the next day. At exactly 7:00 am the second day, empty the bladder using this last voiding to complete the collection.

Refer to specimen requirements for each assay to determine the appropriate preservative. Patients should be given two containers and instructed to avoid direct contact with collection bottles or bags containing preservatives, especially hydrochloric acid. Measure and clearly record the 24-hour volume on the TRF. Submit only the required aliquot of a well-mixed total collection.

Test Menu

A more complete test menu can be found online at Labcorp.com. The online test menu contains test-specific information, including test numbers, CPT codes, specimen requirements, and reference intervals.

Endocrinology Response Tests

The response tests in this section represent established protocols for endocrine stimulation and suppression tests obtained from endocrine literature. Some tests may involve considerable risk to the patient. Response tests should be performed under the supervision of licensed physicians, and in some cases, only in a hospital or specialized patient center where constant medical supervision can be maintained and medications are readily available to counteract possible adverse effects. It should be noted that no test has 100% specificity and 100% sensitivity. Variability is present in patients with the same disease and results obtained must be interpreted in conjunction with the patient's history and clinical examination. Hence, interpretations of laboratory tests are guidelines and not absolutes in many cases.

Contents

Adrenal Stimulation Tests

ACTH Stimulation Test (Cosyntropin)

Purpose

Evaluation of possible primary or secondary adrenal insufficiency, and disorders of adrenal steroid biosynthesis such as Congenital Adrenal Hyperplasia (CAH).[1,6] This test has been used in both pediatric and adult patients.

Rationale

Cosyntropin (ACTH 1-24) consists of the first 24 amino acids of the N-terminal portion of the intact native ACTH molecule (ACTH 1-36). This portion comprises the biologically active region of intact ACTH and is less allergenic than other forms of ACTH. It acts rapidly when bound to the melanocortin-2 receptor (MC2R) of the adrenal cortex initiating synthesis and release of cortisol and its precursors by 30 minutes[2] and a saturating pulse dose is believed to act for at least two hours. The magnitude and duration of response as measured by serum steroid levels may depend on the prior stimulation or suppression of the hypothalamic-pituitary-adrenal axis and the adequacy of drug administration. Normative interpretive data (see accompanying Adrenal Steroid Response to ACTH section) are for serum levels drawn at 60 minutes post-stimulation and assume supraphysiological drug levels causing maximal cortical stimulation. The half-life of Cosyntropin is about 15 minutes and, since the drug is not reported to have any direct toxicity, a dose of 15 micrograms/kg up to a full dose of 250 micrograms (for a patient weight of ≥37 kg) will give reliable results with the high likelihood of maximal stimulation; that a maximal cortisol response occurs at 30 minutes post one microgram of ACTH (1-36) has been reported, but in that report, the response waned by 60 minutes.[4]

Testing strategies should be tailored to the patient's age and suspected diagnosis

- **ACTH deficiency** is suspected. Atrophy of the adrenal cortex due to ACTH deficiency may result in lack of a significant cortisol response to single-pulse ACTH administration; the aldosterone response usually remains normal due to its synthesis in the zona glomerulosa which is orchestrated by the renin-angiotensin system not ACTH. Repeated ACTH stimulation may be necessary to judge the latent potential for synthesis of cortisol by the adrenal zonas fasciculate and reticularis. An unstimulated morning cortisol level of 15 µg/dL suggests an intact hypothalamic-pituitary-adrenal axis.[7] Metyrapone and insulin tolerance tests (ITT) have been considered the gold standards for evaluation of the hypothalamic-pituitary-adrenal axis, although a low-dose (one microgram of Cosyntropin) can be considered instead of the ITT.[4,5,7-9]
- **Disorder of steroid biosynthesis (CAH)** is suspected. A **baseline** and **60-minute post-stimulation** CAH Comprehensive Screen [Androstenedione, 11-Desoxycortisol (Specific Compound S), Cortisol, DHEA, Deoxycorticosterone (DOC), 17-OH-Prgnenolone, Progesterone,17-OH-Progesterone, and Testosterone] for initial diagnosis. Abnormally high or low individual results, along with the clinical assessment, will guide diagnosis and treatment. Some genetic abnormalities, such as P450 Oxido Reductase Deficiency, may be difficult to diagnose by these biochemical means.[1]
- **Primary adrenal insufficiency** is suspected. If in an adrenal insufficiency, crisis stimulation testing can be delayed until patient stabilization, although usually an ACTH level and cortisol measurement can be drawn before hydrocortisone administration.
- **Cortical suppression by chronic corticosteroid administration** is suspected. If corticosteroid had been recently administered, a hypoadrenal crisis is unlikely and a routine ACTH stimulation test can often proceed at the physician's discretion. Modulation of the synthetic response may be blunted even though a supraphysiological dose is administered.

In all testing scenarios, a baseline ACTH should be drawn. Baseline expected values for adrenal steroids are indicated, by age, in the Adrenal Steroid Response to ACTH section.

Procedure

- The patient may have a fat-free meal prior to testing.
- Although it may make more physiologic sense to perform the test during the nadir of endogenous ACTH production (around midnight), it is usually performed at 8:00-9:00 AM for ease of collection and comparison to normative data which was collected during that time-frame.
- Blood is drawn for baseline studies and the ACTH (1-24) administered intramuscularly or intravenously.
  - If intramuscularly, 250 ug of Cosyntropin for patient weight of ≥37 pounds.
  - If intravenously, dilute the Cosyntropin in 2 to 5 mL of normal saline and inject over 2 minutes.
- Samples for analysis are collected 60 minutes after administration of the Cosyntropin.

Specimen Requirements

- ACTH: Recommended 1.0 mL EDTA plasma. Minimum: 0.3 mL. Collect into iced EDTA tube for ACTH. **Separate immediately.** Store and ship frozen in a plastic vial. Submit separate plasma vials for each analysis.
- Cortisol: Recommended 0.5 mL serum. Minimum: 0.1 mL. Separate within one hour. Store and ship frozen in plastic vial. Submit separate serum vials for each analysis.
- Additional steroid hormones (CAH screen): 1.0 ml serum, separate within one hour. Store and ship frozen in plastic vial.

Please note: Providing the minimum volume does not allow for repeat analysis.

Interpretation

A rise from the baseline of at least 7 to 10 µg/dL of cortisol, reaching at least 18 µg/dL at 60 minutes post-stimulation, effectively rules out primary adrenal insufficiency and suggests that adrenal suppression is minimal. A blunted or absent response suggests some level of secondary adrenal insufficiency (cortical atrophy or significant suppression). If a subnormal response is obtained with an elevated baseline ACTH level, the patient has primary adrenal insufficiency or a form of ACTH unresponsiveness. A subnormal response with a low baseline ACTH level suggests CRF (corticotrophin releasing factor) and/or ACTH deficiency of hypothalamic and/or pituitary origin. Prior administration of estrogens, spironolactone, cortisone and hydrocortisone (cortisol) can all interfere with the ACTH stimulation test by causing abnormally high baseline cortisol levels.

In children with CAH, a specific enzyme deficiency is reflected in accumulation of steroid intermediates along the synthetic pathway of cortisol prior to, or parallel to the blocked enzymatic step. The levels of intermediates between cholesterol and cortisol, and on the alternative pathways leading to testosterone and aldosterone production, are different in newborns than in older children and adults; normal levels at various ages are listed in the Adrenal Steroid Response to ACTH section to aid in interpretation of the test results.

References

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2. Spiga F, Liu Y, Aguilera G, Lightman SL. Temporal effect of adrenocorticotrophic hormone on adrenal glucocorticoid steroidogenesis: involvement of the transducer of regulated cyclic AMP-response element-binding protein activity. *J Neuroendocrinol.* 2011; 23(2):136-142. PubMed 21083631.
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Cushing's Disease and Cushing's Syndrome Screening Tests

Rationale

The Endocrine Society has published guidelines for screening and diagnosis of Cushing's disease and Cushing's syndrome.[1] Cushing's syndrome is used to describe all causes of excess glucocorticoid, while Cushing's disease is reserved for the pituitary dependent form of the disease caused by excess ACTH. When clinical signs and symptoms of excess cortisol are present, and exogenous glucocorticoid use has been excluded, screening is appropriate. One screening test from the following is recommended: 24-hour urine cortisol with creatinine, late-night salivary cortisol, 1 mg overnight or 2 mg 48-hour dexamethasone suppression test. Additional testing may be needed following treatment to assess effectiveness and recurrence.

24-hour urine cortisol and late-night salivary cortisol – Endocrine Society recommendation to provide at least two measurements[1]

Procedure

Instruct the patient to collect a 24-hour urinary free cortisol (UFC) in a container with boric acid preservative, which may be provided by the laboratory. Only complete and accurately collected 24-hour urines will be useful due to the diurnal variation of cortisol levels and excretion. The creatinine amount in the collection is a useful indicator of the accuracy of the collection; the ratio of UFC/creatinine provides the most useful information. UFC/creatinine ratio should not be used on spot urines or samples that are not 24-hour collections due the strong diurnal variation of cortisol. UFC has been used for diagnosis of Cushing's syndrome in children.[2] Older diagnostic criteria may be based on nonspecific assays. Labcorp has developed method-specific reference ranges for this analyte. The laboratory-specific reference range should be used, and any patient with an abnormal result should be further evaluated.

Interpretation

In general, urine free cortisol/creatinine that is four times the upper limit of normal is diagnostic of Cushing's disease and Cushing's syndrome except in cases involving patients with Pseudo-Cushing's syndrome, i.e., patients with psychiatric disorders, morbid obesity, poorly-controlled diabetes, etc. A positive test requires additional studies to differentiate the causes of Cushing's syndrome and Cushing's disease.

Late-Night Salivary Cortisol

Procedure

Two separate measurements, collected on two nights, are recommended for screening. Labcorp has developed method-specific reference ranges for this analyte. The laboratory-specific reference range should be used, and any patient with an abnormal result should be evaluated further.

Interpretation

Recently, Dr. Rachel Gafni and colleagues at the National Institutes of Health (NIH) have demonstrated that a midnight salivary cortisol of greater than 0.27 µg/dL identified patients with Cushing's disease and Cushing's syndrome. The diagnostic accuracy of midnight salivary cortisol and 24-hour urine free cortisol/creatinine appear to be similar.[3]

Single Dose Dexamethasone Suppression Test

Rationale

The single-dose dexamethasone test is also used in screening patients suspected of having Cushing's disease and Cushing's syndrome. This test has not been utilized routinely in children and has not been well standardized in children. Hence, its sensitivity and specificity in children has not been established.

In normal subjects, administration of this synthetic glucocorticoid inhibits ACTH secretion and subsequent cortisol production by negative feedback to the hypothalamus and pituitary. In patients with Cushing's disease and Cushing's syndrome, effective suppression of cortisol secretion does not occur with glucocorticoid administration because of continuing autonomous production of ACTH or cortisol. Dexamethasone is the preferred glucocorticoid for this test because it does not interfere with the measurement of cortisol or its urinary metabolites. Dexamethasone (1 mg for adults) is administered in the late evening, between 11 PM and midnight, in order to block the early morning ACTH surge. The single-dose dexamethasone test is valuable in screening for Cushing's disease and Cushing's syndrome because a normal response showing sufficiently decreased cortisol levels essentially rules out this diagnosis.

Special conditions

The response to dexamethasone is blunted in pregnancy, but late-night salivary cortisol and UFC are useful as screening tests. Urine cortisol and salivary cortisol are understood to reflect free (unbound) cortisol and thus are not affected by elevated transcortin (CBG) levels found in pregnancy and estrogen replacement.[1]

Single-Dose Overnight Dexamethasone Suppression Test

Procedure

- Draw for ACTH and cortisol at 8:00 AM
- Give dexamethasone orally at 11:00 PM, 20 µg/kg up to 1 mg (or 0.3 mg/m2).
- Draw blood for serum ACTH and cortisol at 8:00 AM the following morning prior to food ingestion.

Specimen Requirements

- ACTH: Recommended 1.0 mL EDTA plasma. Minimum: 0.3 mL. Collect into iced EDTA tube for ACTH. **Separate immediately.** Store and ship frozen in a plastic vial.
- Cortisol: Recommended 0.5 mL serum. Minimum: 0.1 mL. Separate within one hour. Store and ship frozen in plastic vial.

Please note: Providing the minimum volume does not allow for repeat analysis.

48-hour 2mg/day dexamethasone test Dexamethasone Suppression Test

Procedure

- Draw baseline cortisol.
- Give dexamethasone over 48 hours in doses of 0.5 mg, beginning at 9:00 AM on day 1, at 6-hour intervals for a total of eight doses in the 48-hour period. Serum cortisol is measured six hours after the last dose of dexamethasone.

Specimen Requirements

- Same as for the single-dose dexamethasone suppression test.

Interpretation

A serum cortisol level greater than 1.8 µg/dL after the single-dose overnight dexamethasone is considered a positive test.[5] Previously, experts recommended that only levels greater than 5 µg/dL were positive, however this leads to a loss of sensitivity of the screening test.[1] False-positive tests can occur in obese patients and those who have had a poor night's sleep. In patients under acute emotional or physical stress, a serum dexamethasone level may be ordered to confirm compliance and absorption of dexamethasone. If Cushing's disease or Cushing's syndrome is suspected, a 24-hour urine for free cortisol and/or a 2mg-2 day dexamethasone test should be performed to confirm the diagnosis and help differentiate the etiology. The ACTH level may be helpful since lack of suppression of cortisol with an inappropriately elevated ACTH suggests an ACTH-dependent etiology-Cushing's disease or perhaps Ectopic ACTH Syndrome. A lack of suppression with a low or un-measurable ACTH level suggests an adrenal tumor, nodular hyperplasia, or ectopic cortisol production. The standard 7-day dexamethasone test is no longer part of the recommendations.

A serum cortisol level greater than 1.8 µg/dL after the 48-hour 2mg/day dexamethasone suppression is considered a positive test. Caveats are similar to the single dose dexamethasone test above.

Limitations and Pseudo-Cushing's Syndrome

Pseudo-Cushing's syndrome is a term used to describe hypercortisolism due to alcohol or depression, or obesity.[6] In addition, phenytoin, phenobarbitone, carbamazepine, rifampin, and alcohol induce hepatic enzymatic clearance of dexamethasone, and the accelerated clearance of dexamethasone may lead to a false-positive result for patients on these drugs because lower levels of dexamethasone result in reduced feedback to the hypothalamic-pituitary axis. Tegretol has also been shown to interfere with dexamethasone suppression. Acromegaly and Grave's disease may also produce false positive results. If a false-positive result is suspected, the test should be repeated or a different test should be selected.

References
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2. Magiakou MA, Mastorakos G, Oldfield EH, et al. Cushing's Syndrome in Children and Adolescents. *N Engl J Med.* 1994; 331(10):629-636. PubMed 8052272
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4. Vilar L, Freitas MC, Lima LH, et al. Cushing's Syndrome In Pregnancy: an Overview. *Arq Bras Endocrinol Metabol*. 2007; 51(8):1293-1302. PubMed 18209867
5. Guignat L, and Bertherat J. The diagnosis of Cushing's syndrome: an Endocrine Society Clinical Practice Guideline: commentary from a European perspective. *Eur J Endocrinol*. 2010; 163(1):9-13. PubMed 20375177.
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Additional Resources

Nieman LK, Biller BM, Findling JW, et.al. Treatment of Cushing's Syndrome: An Endocrine Society Clinical Practice Guideline. *J Clin Endocrinol Metab*. 2015; 100: 2807–2831. PubMed 26222757

Adrenal Insufficiency Screening Tests

Single-Dose Metyrapone Test

Purpose

Assessment of the functional integrity of the pituitary-adrenal axis. An 8:00 AM to 9:00 AM serum cortisol measurement greater than 8 µg/dL indicates adequate adrenal production for patients not on replacement glucocorticoid. See section on ACTH stimulation tests.

Rationale

Metyrapone may not be available in all markets.

11-deoxycortisol (Compound S) is converted to cortisol by the adrenal enzyme, 11-beta-hydroxylase, which is selectively inhibited by metyrapone. When metyrapone is given to a normal subject, cortisol levels fall due to the inhibition, stimulating ACTH production by the pituitary. The increased ACTH levels in turn stimulate the adrenal gland to produce additional 11-deoxycortisol because cortisol production is blocked. Since 11-deoxycortisol exerts no feedback control on ACTH production, ACTH continues to rise in response to metyrapone in the normal individual and results in a sharp increase in the serum 11-deoxycortisol level.

The changes in 11-deoxycortisol levels can be measured most reliably in serum. The magnitude of the increase over baseline far exceeds that observed when measuring urinary metabolites.

Procedure (adults[1] or children[2])

- Draw baseline ACTH, cortisol, and 11-deoxycortisol.
- Give metyrapone (30 mg/kg; maximum 2.0 g) orally in a single dose at midnight. Milk or a light snack should be given at the same time to decrease the nausea that often occurs with this drug.
- At 8:00 AM the following morning, draw blood for plasma ACTH, serum cortisol, and 11-deoxycortisol. Plasma may be used for all tests.

Specimen Requirements

- 11-Desoxycortisol (Compound S, Metyrapone Test): Recommended 0.5 mL serum. Minimum: 0.2 mL. Separate within one hour. Store and ship frozen in plastic vial.
- ACTH: Recommended 1.0 mL EDTA plasma. Minimum: 0.3 mL. Collect into iced EDTA tube for ACTH. **Separate immediately.** Store and ship frozen in a plastic vial.
- Cortisol: Recommended 0.5 mL serum. Minimum: 0.1 mL. Separate within one hour. Store and ship frozen in plastic vial.

Please note: Providing the minimum volume does not allow for repeat analysis.

Interpretation

The normal response is an increase in serum 11-deoxycortisol to levels greater than 7 µg/dL with a rise in ACTH to greater than 100 pg/mL. ln patients with primary or secondary adrenocortical insufficiency, 11-deoxycortisol generally will be less than 5 µg/dL.[3] Alternatively, combined cortisol plus desoxycortisol level over 15.5 µg/dL is considered adequate response.[1]

If a normal rise in 11-deoxycortisol is not observed, the ACTH and cortisol level should be checked to confirm adequate metyrapone blockade. A cortisol level below 5 µg/dL usually indicates adequate suppression. Cortisol levels above 10 µg/dL in the presence of an inadequate rise in 11-deoxycortisol may indicate ineffective suppression and the test should be repeated. An abnormal metyrapone test does not differentiate primary adrenal insufficiency (adrenal gland impairment) from secondary adrenal insufficiency (pituitary impairment). However, secondary adrenal insufficiency is strongly suggested in those who respond to ACTH stimulation tests but not to metyrapone, and in those who do not have a rise in ACTH with adequate metyrapone blockade. Patients with primary adrenal insufficiency generally have markedly-elevated baseline plasma ACTH levels, as well as elevated plasma ACTH levels post-metyrapone.

Limitations and Notes

Metyrapone may produce gastric irritation and consequent nausea and vomiting. Thus, it should be given with milk or food to prevent vomiting. Caution should be observed when administering metyrapone to patients suspected of having primary adrenal insufficiency since there is a risk of adrenal crisis in these cases. Patients of primary adrenal insufficiency generally have markedly elevated baseline plasma ACTH levels. All patients should be tested in the hospital and be observed closely during the test so that supportive measures can be instituted if the patient develops hypotension or vomiting. After the test, one can consider hydrocortisone coverage for the day depending on the patient's clinical status. Patients on Dilantin® or other drugs that increase hepatic P450 enzyme activity generally have subnormal increases in 11-deoxycortisol levels after metyrapone administration due to increased metabolism of the drug. An abnormal response may also be seen following glucocorticoid therapy because of suppression of the hypothalamic pituitary-adrenal axis.

Dilantin is a registered trademark of Pfizer, Inc.

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Pituitary Stimulation Tests for Children (for adults, refer to subsequent section)

Growth Hormone Stimulation Tests

General Comments

Stimulation tests are useful for the evaluation of a child with suspected growth hormone deficiency.[1,2] They are also widely used to evaluate pituitary function and serve as indicators of destructive lesions of the pituitary or hypothalamus. Stimulation tests are often required because basal growth hormone is usually low and fluctuates periodically throughout the day. Its secretion can be stimulated physiologically (during sleep or after strenuous exercise) or pharmacologically. The measurement of serum IGF-1 and/or IGFBP-3, or sleep or exercise tests, are employed by clinicians as screening tests for growth hormone deficiency. It must be emphasized that growth hormone stimulation testing should be considered in patients who have auxological, clinical, and chemical criteria, strongly suggesting the presence of growth hormone deficiency.

Some investigators[3] have suggested that, in a child with no other indication of systematic or endocrine disease, the presence of low levels of IGF-1 and IGFBP-3 along with auxological data consistent with growth hormone deficiency are adequate for diagnosis, or at least no worse than the results obtained by stimulation tests for growth hormone deficiency. However, general belief is that definitive testing for growth hormone deficiency requires the use of pharmacologic agents.[4-6]

The most popular and reliable of these agents are insulin, arginine, glucagon, L-dopa/carbidopa, and clonidine. Although all can elicit growth hormone increases in a majority of patients, a normal child may fail to respond appropriately to any one of these pharmacologic stimuli because there is a refractory period in GH secretion following a normal GH pulse. Therefore, an inadequate response to a single stimulation test in a child who does not have a documented organic cause (such as craniopharyngioma, septo-optic dysplasic, post-CNS tumor therapy,CNS irradiation, or congenital panhypopituitarism, etc.) cannot be considered diagnostic of growth hormone deficiency. An inadequate response to at least two stimulation tests, along with auxologic data consistent with growth hormone deficiency, are recommended to confirm a diagnosis. Because of the high frequency of false-positive results, some investigators have found that administering 40 µg/m^2 ethinyl estradiol/day, divided into three doses with meals for two days,[7] or micronized estradiol valerate 1 mg (up to 20 kg weight), 2 mg (over 20 kg) nightly for three days prior to the GH stimulation test[8] can facilitate a growth hormone response.

Priming with sex steroids is said to be especially helpful in short patients with delayed adolescence since these patients could be considered to be functionally partially growth-hormone-deficient due to a lack of endogenous sex steroids. Conversely, there are rare circumstances, such as radiation to the brain, where some pharmacologic stimulation (arginine) can evoke a normal response even though others (insulin) do not, and the clinical and physiologic state suggests growth hormone secretion is insufficient.[9,10]

Finally, many drugs, non-pituitary illnesses, and poor nutritional states may alter either the basal level or maximal response to these tests. Obesity or hypothyroidism may cause decreased growth hormone responses to stimulatory agents. Hypothyroidism must be corrected before pituitary function can be reliably evaluated for growth hormone. A recent study in children showed that higher BMIs, even within the normal range, could lead to the over diagnosis of GHD.[11] Certainly, the diagnosis of growth hormone deficiency should be viewed with caution in the patient in whom no evidence of an abnormality in the hypothalamus or pituitary is seen on MRI.

Insulin-Induced Hypoglycemia Test

Purpose

Assessment of growth hormone secretion and the hypothalamic-pituitary-adrenal axis.

Rationale

Hypoglycemia can promote the release of a number of hormones, including growth hormone, ACTH (resulting in cortisol secretion), and prolactin. The administration of insulin to the normal child will lower blood glucose levels and generally, but not invariably, lead to a compensatory rise in growth hormone and cortisol secretion.

Procedure

The following is a synthesis of the procedure most commonly recommended:

- The test is usually performed in the morning after an overnight fast, or if the patient is an infant, after a four-hour fast.
- An indwelling intravenous (IV) catheter with keep open flow of non-glucose-containing solution of physician's choice or a butterfly with a heparin lock should be placed and maintained patent throughout the entire test so that the test can be terminated promptly, if necessary, with IV glucose. The IV line also may be used for administering insulin, and obtaining blood samples throughout the test.
- Baseline 0-minute blood samples for growth hormone glucose and cortisol should be drawn.
- If baseline glucose is greater than 70 mg/dL, the literature recommends injecting regular insulin, 0.075 or 0.1 units/kg intravenously. When panhypopituitarism is strongly suspected, 0.05 units/kg IV is recommended. For accuracy in administering insulin, one can dilute insulin 1:10 with sterile normal saline prior to injection.
- Obtain blood samples for glucose and growth hormone at 15, 30, 45, 60, 90, and 120 minutes after insulin injection. A 60-minute sample for cortisol should be obtained to evaluate adrenal response to hypoglycemic stimulus as a measure of adrenal reserve.
- During the test, the patient should be continuously assessed and monitored by a physician in attendance. A solution of 25% glucose in water should be available for IV administration, if needed. At the termination of the test, a meal or intravenous glucose in water should be given immediately.
- This test may be performed in sequence following an arginine infusion test.

Specimen Requirements

- Growth Hormone: Recommended 1.0 mL serum for each determination. Minimum: 0.4 mL. Separate within one hour. Store and ship frozen in plastic vial.
- Glucose: Recommended 0.5 mL serum for each determination. Minimum: 0.1 mL. Collect in a tube with NaF. Separate within one hour. Store and ship frozen in a plastic vial.
- Cortisol: Recommended 0.5 mL serum for each determination. Minimum: 0.1 mL. Separate within one hour. Store and ship frozen in a plastic vial.

Please note: Providing the minimum volume does not allow for repeat analysis.

Interpretation

Hypoglycemia is a potent stimulus to secretion of GH.[19] It is important that adequate hypoglycemia be achieved to provide the stimulus necessary for growth hormone secretion. Serum glucose levels should fall to values less than 45 mg/dL. Generally, patients will demonstrate mild symptoms of hypoglycemia during the test (nervousness, sweating or tachycardia). However, if more severe symptoms of hypoglycemia develop (ex: somnolence, seizures or other disturbing behavior), recommendations are for stopping the test and administering glucose through the IV according to physician experience in treating hypoglycemia.

The assessment of GH secretory capacity is complicated because of the episodic nature of GH release from the pituitary. Basal GH levels can exhibit considerable variability throughout a 24-hour period, thus limiting their clinical utility. Alternatively, measurement of GH response to various stimuli has commonly been used to improve the diagnostic assessment of GH secretion.

GH response to provocative stimuli among normal individuals, however, is highly variable. Response values greater than 10 ng/mL have historically been considered to reflect normal GH secretory function, while values below 10 ng/mL have been considered to indicate some degree of GH deficiency. However, it should be noted that this limit is arbitrarily derived given the differences in assay sensitivities and the variable responses to the stimuli seen in normal stature children.[12] In addition, the cut off varies from country to country according to the value approved by the regulatory agency which approved GH. Nevertheless, some clinicians utilize 7 ng/mL as the minimal normal response in children and others, 10 ng/mL as the cut-off. In addition, recent literature suggests that, of adolescents in the transition phase with idiopathic isolated GHD diagnosed in childhood, between 60% and 70% no longer have GHD upon retesting.[13]

Precautions

This test is relatively safe and reliable if adequate precautions are observed. Patients will usually have mild symptoms of hypoglycemia during the procedure. Indeed, the absence of any symptoms suggests an inadequate test. However, severe hypoglycemic reactions can occur. Constant medical attention is required during the entire procedure. Preparations for adverse reaction should be made prior to testing and hypoglycemia reversed immediately if confusion, hypotension, loss of consciousness or seizures occur. This test is contraindicated in patients with a history of hypoglycemia, heart disease or seizure disorders.

L-Dopa/Carbidopa Test

Purpose

Assessment of growth hormone secretion.

Rationale

See General Comments.
Levo-dopa, a dopamine precursor that crosses the blood-brain barrier, has been used to evaluate growth hormone secretory capacity. Although the mechanism of action is not entirely known, it appears that L-dopa is capable of promoting growth hormone release by either a direct effect on the pituitary, or more likely through stimulation of hypothalamic growth hormone-releasing hormone. It has also been shown to stimulate the release of ACTH and cortisol.[14]

The manufacture of L-Dopa has been curtailed in the US, making it unavailable for growth hormone stimulation testing. However, a combination of L-Dopa/carbidopa (in three mixed-dose combinations) is available under the commercial name of Sinemet® (Merck). Carbidopa decreases the activity of dopamine carboxylase, resulting in a longer half-life of circulating L-Dopa and hence, greater potency. This combination alone, or along with propranolol priming, has been used for growth hormone stimulation in children,[14,15] and some literature suggests it may be more effective that L-Dopa alone.[16]

Procedure

This test is usually performed in the morning after an overnight fast. Draw a baseline blood sample for growth hormone from an indwelling venous catheter or butterfly kept open with a heparin lock and maintained patent throughout the entire test.

- Administer L-Dopa/carbidopa orally, 125 mg/12.5 for body weight less than 30 lbs, 250 mg/25 for body weight over 30 lbs.
- Obtain blood samples for growth hormone at 30, 60, 90, and 120 minutes.
- Some investigators recommend pre-treatment with propranolol, 0.75 mg/kg to a maximum of 40 mg, given orally 1 hour prior to L-Dopa/carbidopa administration. This significantly reduces the incidence of false-negative responses. Propranolol should not be given to children with asthma, diminished cardiac reserve, a history of hypoglycemia or extreme thinness. If used, the indwelling catheter or butterfly should be placed at this time and serum glucose should be checked prior to propranolol administration, and it is recommended that it not be given unless the glucose level is normal (about 70 mg/dL).

Specimen Requirements

- Growth Hormone: Recommended: 1.0 mL serum for each determination. Minimum: 0.4 mL serum. Separate within one hour. Store and ship frozen in plastic vial.

Please note: Providing the minimum volume does not allow for repeat analysis.

Interpretation

The assessment of GH secretory capacity is complicated because of the episodic nature of GH release from the pituitary. Basal GH levels can exhibit considerable variability throughout a 24-hour period, thus limiting their clinical utility. Alternatively, measurement of GH response to various stimuli has commonly been used to improve the diagnostic assessment of GH secretion. GH response to provocative stimuli among normal individuals, however, is highly variable. Response values greater than 10 ng/mL have historically been considered to reflect normal GH secretory function, while values below 10 ng/mL have been considered to indicate some degree of GH deficiency. However, it should be noted that this limit is arbitrarily derived. A significant percentage of normal controls exhibit response values well below this 10 ng/mL limit. Some clinicians consider 7 ng/mL to be the minimal normal response to provocative stimuli in children and adolescents. In addition, recent literature suggests that, of adolescents in the transition phase with idiopathic isolated GHD diagnosed in childhood, between 60% and 70% no longer have GHD upon retesting.[13]

Patients should be tested while fasting since hyperglycemia will blunt the normal response. As with other tests of growth hormone secretory capacity, a normal patient occasionally will not respond to L-dopa/carbidopa. Therefore, an additional stimulatory test should be used to confirm the diagnosis of growth hormone deficiency.

Precautions

Generally, side effects are minor when present. These include nausea or vomiting, vertigo and mild headaches.

Clonidine Test

Purpose

Assessment of growth hormone secretion.

Rationale

Clonidine, an alpha adrenergic stimulus, has been used since 1979[17] to evaluate growth hormone secretory capacity. Although the mechanism of action is not entirely known, it appears that clonidine is capable of promoting growth hormone release by a direct effect on the stimulation of hypothalamic growth hormone-releasing hormone.[18]

Procedure

- This test is usually performed in the morning after an overnight fast.
- A baseline blood sample for growth hormone should be obtained from an indwelling venous catheter or butterfly kept open with a heparin lock and maintained patent throughout the entire test.

- Administer Clonidine orally, 0.15 mg/m^2.
- Obtain blood samples for growth hormone at 30, 60, 90, and 120 minutes.

Specimen Requirements

- Growth Hormone: Recommended: 1.0 mL serum for each determination. Minimum: 0.4 mL serum. Separate within one hour. Store and ship frozen in plastic vial.

Please note: Providing the minimum volume does not allow for repeat analysis.

Interpretation

The assessment of GH secretory capacity is complicated because of the episodic nature of GH release from the pituitary. Basal GH levels can exhibit considerable variability throughout a 24-hour period, limiting their clinical utility. Alternatively, measurement of GH response to various stimuli has commonly been used to improve the diagnostic assessment of GH secretion. GH response to provocative stimuli among normal individuals, however, is highly variable. Response values greater than 10 ng/mL have historically been considered to reflect normal GH secretory function, while values below 10 ng/mL have been considered to indicate some degree of GH deficiency. However, it should be noted that this limit is arbitrarily derived. A significant percentage of normal controls exhibit response values well below this 10 ng/mL limit. Some clinicians use 7 ng/mL as the lower limit of normal response in children and adolescents. In addition, recent literature suggests that, of adolescents in the transition phase with idiopathic isolated GHD diagnosed in childhood, between 60% and 70% no longer have GHD upon retesting.[13]

Patients should be tested while fasting since hyperglycemia will blunt the normal response. As with other tests of growth hormone secretory capacity, a normal patient occasionally will not respond to clonidine. Therefore, an additional stimulatory test should be used to confirm the diagnosis of growth hormone deficiency.

Precautions

Generally, side effects are minor when present. These include hypotension and somnolence. Therefore, it is prudent to keep the patient supine during the test to prevent consequences that could result from hypotension and somnolence.

Arginine Infusion Test

Purpose

Assessment of growth hormone secretion.

Rationale

See General Comments.
It has been shown that a variety of amino acids given intravenously can stimulate growth hormone secretion. Arginine is the most commonly used of these preparations and may be given alone or sequentially with insulin.[20] Its mechanism of action is not entirely clear, but may involve adrenergic stimulation of the hypothalamus.

Procedure

- This test is usually performed in the morning after an overnight fast.
- An IV or indwelling butterfly needle with or without an indwelling catheter and locked with heparin should be placed and a baseline blood sample for growth hormone and glucose obtained.
- Infuse a sterile 10% solution of arginine monohydrochloride 0.5 g/kg (maximum dose 30 g) intravenously (IV) over 30 minutes.
- Obtain blood samples for growth hormone at 30, 60, 90, and 120 minutes. These may be obtained from the indwelling IV line that can be kept open with normal saline.
- Pre-treatment with estrogen two days prior to the test or propranolol at one hour prior to the test may enhance the growth hormone response and decrease the percentage of false-negative response.
- This test may be performed in sequence with insulin or L-dopa after the arginine test. Please note: Studies using L-dopa/carbidopa stimulation after arginine infusion have not been published. Hence, the efficacy and possible side effects are not documented.

Specimen Requirements

- Growth Hormone: Recommended: 1.0 mL serum for each determination. Minimum: 0.4 mL serum. Separate within one hour. Store and ship frozen in plastic vial.

Please note: Providing the minimum volume does not allow for repeat analysis.

Interpretation

The assessment of GH secretory capacity is complicated because of the episodic nature of GH release from the pituitary. Basal GH levels can exhibit considerable variability throughout a 24-hour period, thus limiting their clinical utility. Alternatively, measurement of GH response to various stimuli has commonly been used to improve the diagnostic assessment of GH secretion. GH response to provocative stimuli among normal individuals, however, is highly variable. Response values greater than 10 ng/mL have historically been considered to reflect normal GH secretory function, while values below 10 ng/mL have been considered to indicate some degree of GH deficiency. Conversely, there are rare circumstances, such as radiation to the brain, where some pharmacologic stimulation (specifically arginine) can evoke a normal response even though others (insulin) do not, and the clinical and physiologic state suggest growth hormone secretion is insufficient.[9,10] Thus, while arginine is used in children up to and including the transition phase, with that caveat, it is not recommended for use in adults.[21]

Precautions

Generally, this is a safe test with few side effects. Arginine stimulates the release of insulin and glucagon. Serum glucose usually remains normal, but hypoglycemia can occur. Arginine should be given with caution to patients with severe hepatic or renal disease and in patients who are acidotic since arginine monohydrochloride infusion induces acidosis.

GHRH- Arginine Infusion Test (GHRH is no longer commercially available in the United States)

Glucagon Stimulation Test

Purpose

Assessment of growth hormone secretion.

Rationale

See General Comments.
Glucagon has been shown to increase growth hormone release and thus has been used as a pharmacologic stimulus for growth hormone testing. It is relatively safe, especially in neonates who may have low glucose levels secondary to their multiple pituitary hormone deficiencies.

Procedure

- This test is usually performed after an overnight fast or after a two- to three-hour fast in the neonate or infant with low blood sugar.
- Start IV or a butterfly needle without an indwelling catheter and keep open with normal saline or a heparin lock (no glucose). Draw baseline glucose and growth hormone samples.
- Give Glucagon 0.03 mg/kg (maximum dose 1 mg), intra muscular or subcutaneous injection.
- Obtain blood samples for glucose and growth hormone at 15, 30, 60, 90, 120, 150, and 180 minutes. Note that this timeline is longer than the other stimulation tests described because the rise in GH is variable and the peak is delayed compared to other stimuli.

Specimen Requirements

- Growth Hormone: Recommended: 1.0 mL serum for each determination. Minimum: 0.4 mL serum. Separate within one hour. Store and ship frozen in plastic vial.

Please note: Providing the minimum volume does not allow for repeat analysis.

Interpretation

The assessment of GH secretory capacity is complicated because of the episodic nature of GH release from the pituitary. Basal GH levels can exhibit considerable variability throughout a 24-hour period, thus limiting their clinical utility. Alternatively, measurement of GH response to various stimuli has commonly been used to improve the diagnostic assessment of GH secretion. GH response to provocative stimuli among normal individuals, however, is highly variable. Response values greater than 10 ng/mL have historically been considered to reflect normal GH secretory function, while values below 10 ng/mL have been considered to indicate some degree of GH deficiency. However, it should be noted that this limit is arbitrarily derived and a significant percentage of normal control children exhibit response values below the 10 ng/mL limit. Hence, some clinicians utilize 7 ng/mL as their minimal normal response to provocative stimuli in children and adolescents. Growth hormone peak values are generally seen at 120 minutes after glucagon and mean values are reported to be higher than those obtained with arginine or insulin-induced hypoglycemia.

Precautions

Generally, glucagon stimulation is a safe response test, as it stimulates the breakdown of glycogen in the liver to glucose and its release with a consequent rise in blood glucose. However, hypoglycemia can occur after the peak glucose rise in the first 30 to 60 minutes and therefore, serum glucose levels should be monitored closely, especially in the neonate with presumed hypopituitarism. The test should be terminated with feeding or IV glucose if the patient becomes symptomatically neuroglycopenia or the serum glucose decreases to less than 45 mg/dL. Nausea, vomiting, and abdominal pain may also occur as a consequence of glucagon administration.

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Gonadotropin-Releasing Hormone (GnRH) Test

Purpose

Assessment of pituitary gonadotropin secretion.

Rationale

Gonadotropin-releasing hormone (GnRH) is a hypothalamic deca-peptide that stimulates secretion of pituitary LH and FSH. This hormone has been synthesized (Factrel®) and can be used to evaluate pituitary gonadotropin reserve. The response to exogenous GnRH in children appears to be dependent upon the amount of previous exposure of the pituitary to endogenous GnRH. In prepubertal children, the hypothalamic neurons that secrete GnRH are very sensitive to feedback inhibition by small amounts of gonadal sex steroids. As children approach puberty, hypothalamic sensitivity is believed to gradually diminish, resulting in increased GnRH secretion. With continued exposure to GnRH, the pituitary becomes more responsive and secretes greater amounts of gonadotropins, particularly LH. Thus, endogenous GnRH probably acts as a self-primer and augments pituitary sensitivity to exogenous GnRH as children approach puberty. This likely accounts for the increased response observed in pubertal and adult patients.

Traditional GnRH testing has employed six samples collected over a two-hour period. Using two-site ICMA LH and FSH assays allows shorter overall test times, fewer samples, and reduced patient costs. A current protocol requires only a single baseline sample and one other sample collection at 30 or 40 minutes following GnRH administration to accurately assess gonadotropic hormone secretion, although multiple samples over one hour may have a higher degree of sensitivity.

Procedure

- Draw blood for baseline LH and FSH at 0 minutes.
- Give GnRH (Factrel®), 100 ug maximum, by rapid (less than 5 seconds) intramuscularly injection or subcutaneously.
- Draw blood for LH and FSH at 30 to 40 minutes after GnRH administration for short test and at 20, 40, and 60 minutes for the standard test.

Specimen Requirements

- Luteinizing Hormone (LH): Recommended: 1.0 mL serum for each determination. Minimum: 0.3 mL serum. Separate within one hour. Store and ship frozen in plastic vial.
- Follicle-Stimulating Hormone (FSH): Recommended: 1.0 mL serum for each determination. Minimum: 0.3 mL serum. Separate within one hour. Store and ship frozen in plastic vial.

Please note: Providing the minimum volume does not allow for repeat analysis.

Interpretation

The data obtained using new two-site immunoassays have resulted in a major improvement in the value of this stimulation test. Using these assays, basal LH values are very low in prepubertal children (<0.3) and generally remain below 5 mIU/mL after GnRH stimulation. Basal LH values increase from 10- to 40- fold during puberty, and response values after GnRH in normal pubertal children are usually above 8 mIU/mL with levels between 6-8 mIU/mL being “transitional” values. It should be noted that these values are applicable to children more than two years of age. Normal children less than two years of age have a more robust LH response to GnRH than older prepubertal children.

In normal prepubertal children over two years of age, there is a diminished LH response to GnRH, and the magnitude of the FSH rise is generally greater than that of LH. With the onset of puberty, there is a brisk LH response to exogenous GnRH with LH peak levels being higher than FSH. Responses to GnRH in females usually show significant menstrual cycle variability with greater stimulation of LH observed during the luteal phase. The GnRH test is useful for evaluation of both precocious and delayed puberty. However, the GnRH test does not discriminate well between patients with constitutional delay and those with hypogonadotropic hypogonadism. Because of the variability of responses observed in normal prepubertal and pubertal children, there is some overlap in gonadotropin values obtained after GnRH in these two groups.

The GnRH test has not proven valuable in differentiating between pituitary and hypothalamic disorders. Absent responses can be seen in patients who have hypothalamic diseases, and conversely, many patients with known pituitary disorders have a normal response to exogenous GnRH. Thus, while the GnRH test can demonstrate that the pituitary is capable of gonadotropin release when stimulated, it cannot differentiate conclusively between pituitary and hypothalamic disorders. The GnRH induced LH release in children with true sexual precocity is in the pubertal range, while it is in the prepubertal range in children with precocious thelarche or adrenarche. During treatment of precocious puberty with GnRH agonists, LH progressively decreases to very low levels and responds minimally to GnRH testing. LH values of <2.0 mIU/mL obtained at 30 to 40 minutes after GnRH stimulation are generally considered indicative of adequate gonadotropin suppression.

LH Response To GnRH Stimulation Procedure

Pubertal Stage		-15	0	30	60	90	120
Prepubertal	Mean	0.08	0.05	2.4	2.2	1.6	1.0
Tanner Stage 1	Range	0.02 - 0.25	0.02 - 0.21	0.8 - 7.0	0.7 - 7.5	0.4 - 5.5	0.20 - 2.9
(n = 21)	SD	0.07	0.07	2.2	2.2	1.6	0.92
Early Puberty	Mean	1.2	1.0	19	15	12	9.8
Tanner Stages 2-3	Range	0.10 - 2.6	0.10 - 2.5	6.1 - 53	6.3 - 38	2.5 - 26.8	2.4 - 17.6
(n = 10)	SD	0.85	0.85	14	10	7.4	5.4
Late Puberty	Mean	2.3	2.4	35	32	24	22
Tanner Stages 4-5	Range	0.8 - 5.0	1.0 - 5.9	10.6 - 67	12.9 - 63	6.8 - 47	6.4 - 58
(n = 10)	SD	1.2	1.3	21	20	13	15
Central Precocious	Mean	-	3.1	35	-	-	-
Puberty*	Range	-	-	-	-	-	-
(n = 15)	SD	-	3.9	24	-	-	-
Central Precocious	Mean	-	0.38	0.58	-	-	-
Puberty - treated	Range	-	-	-	-	-	-
(n = 13)	SD	-	0.18	0.37	-	-	-

*Normal Children: Baseline and 30 minute LH levels correlate and increase progressively during puberty. After GnRH stimulation, LH peaks at 30 minutes to a maximum of 7.5 mIU/mL in normal prepubertal children. In our studies, children under 10 years of age had a mean peak of 1.8 mIU/mL, with a standard deviation of 1.3 mIU/mL (n=17). When prepubertal boys up to 11.2 years of age were included, the mean peak value was 2.7 mIU/mL, with a standard deviation of 2.6 mIU/mL (n=21).

Periodically, Factrel® (GnRH) has not been available to the clinician for GnRH testing. Testing with leuprolide acetate given subcutaneously has been shown to be informative.[1] Similarly, leuprolide acetate testing has been found efficacious in the discrimination between hypogonadotropic hypogonadism and constitutional delay of puberty.[2] Recently it has been suggested that pediatric leuprolide depot therapy in children with precocious puberty can be monitored by measuring LH levels 30 to 60 minutes after the injection of the depot, rather than doing a GnRH stimulation test.[3]

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Pituitary Stimulation Tests for Adults (for pediatrics, refer to previous section)

Growth Hormone Stimulation Tests

General Comments

Growth hormone stimulation tests are useful for the evaluation of an adult who exhibits structural hypothalamic/pituitary disease following radiation, surgery, or trauma, or who has documented loss of other pituitary hormones.[1,2] Many current guidelines find there is little to no de novo onset of idiopathic isolated GHD.[3,4]

Stimulation tests are often required because basal growth hormone is usually low and fluctuates periodically throughout the day. Its secretion can be stimulated physiologically (during sleep or after strenuous exercise) or pharmacologically. The measurement of serum IGF-1 and/or IGFBP-3, can be considered in the screening for GHD, recognizing that they can be influenced by body weight and other medical conditions such as hypothyroidism, illness and cachexia. Idiopathic GHD (IGHD) rarely, if ever, occurs in adults and if IGF is low enough to consider the diagnosis, two tests are recommended.[2] The role of body weight in interpreting GH stimulation tests in adults has been assessed and there is a clear relationship between increasing body weight and blunting of the secretion of GH to stimuli.[5-7]

Adults with childhood onset (CO) GHD who are at the end of their treatment for height and are being considered for transition to adult GHD dosing should be retested for GHD with two stimulation tests unless they have known mutations, documented CNS embryopathic lesions, multiple hormone deficiencies, or irreversible CNS structural damage. For these later groups, cessation of GH therapy for one month and a documented low IGF-I is sufficient.[2]

With the withdrawal of GHRH for use as a secretagogue either alone or in combination with arginine, the current recommendations of both the Endocrine Society and American Association of Clinical Endocrinologists (AACE) and a consortium of global societies[2-4] is for the insulin tolerance test as the test of choice for making the diagnosis of Adult GHD. If it is not possible or there is a medical reason not to perform the insulin tolerance test, then a glucagon stimulation test is recommended.[2,3,8,9] However, a recent publication raises the issue of the safety of glucagon in the elderly.[5] Oral agents are not recommended for the diagnosis in adults.[3,4] The use of arginine alone as a single test is also not recommended in the current adult guidelines for testing, but may be used in non-obese pediatric GHD transitioning to adulthood.[4]

Insulin-Induced Hypoglycemia Test

Purpose

Assessment of growth hormone secretion and the hypothalamic-pituitary-adrenal axis.

Rationale

See General Comments.
Hypoglycemia can promote the release of a number of hormones, including growth hormone, ACTH (resulting in cortisol secretion), and prolactin. The administration of insulin to an adult will lower blood glucose levels and generally, but not invariably, lead to a compensatory rise in growth hormone and cortisol secretion.

Procedure (The following is a synthesis of the procedure most commonly reported in the literature.)

- The test is usually performed in the morning after an overnight fast.
- An indwelling intravenous (IV) catheter with open flow of non-glucose-containing solution of physician's choice or a butterfly with a heparin lock should be placed and maintained patent throughout the entire test so that the test can be terminated promptly, if necessary, with IV glucose. The IV line also may be used for administering insulin, and obtaining blood samples throughout the test.
- 0 time blood samples for growth hormone, glucose, and cortisol should be drawn.
- If baseline glucose is greater than 70 mg/dL, the literature recommends injecting regular insulin, 0.075 or 0.1 units/kg intravenously. When panhypopituitarism is strongly suspected, 0.05 units/kg IV is recommended. For accuracy in administering insulin, one can dilute insulin 1:10 with sterile normal saline prior to injection.
- Obtain blood samples for glucose and growth hormone at 15, 30, 45, 60, 90, and 120 minutes after insulin injection. A 60-minute sample for cortisol should be obtained to evaluate adrenal response to hypoglycemic stimulus as a measure of adrenal reserve.
- During the test, the patient should be continuously assessed and monitored by a physician in attendance. A solution of 25% glucose in water should be available for IV administration, if needed. At the termination of the test, a meal or intravenous glucose in water should be given immediately.

Specimen Requirements

- Growth Hormone: Recommended: 1.0 mL serum for each determination. Minimum: 0.4 mL. Separate within one hour. Store and ship frozen in plastic vial.
- Glucose: Recommended: 0.5 mL serum for each determination. Minimum: 0.1 mL. Collect in a tube with NaF. Separate within one hour. Store and ship frozen in a plastic vial.
- Cortisol: Recommended: 0.5 mL serum for each determination. Minimum: 0.1 mL. Separate within one hour. Store and ship frozen in a plastic vial.

Please note: Providing the minimum volume does not allow for repeat analysis.

Interpretation

It is important that adequate hypoglycemia be achieved to provide the stimulus necessary for growth hormone secretion. Serum glucose levels should fall to values less than 45 mg/dL. Generally, patients will demonstrate mild symptoms of hypoglycemia during the test (nervousness, sweating or tachycardia). However, if more severe symptoms of hypoglycemia develop (eg, somnolence, seizures or other disturbing behavior), recommendations are to stop the test and administer glucose through the IV according to physician experience in treating hypoglycemia.

The assessment of GH secretory capacity is complicated because of the episodic nature of GH release from the pituitary. Basal GH levels can exhibit considerable variability throughout a 24-hour period, thus limiting their clinical utility. Alternatively, measurement of GH response to various stimuli has commonly been used to improve the diagnostic assessment of GH secretion.

GH response to provocative stimuli among normal individuals, however, is highly variable. In adults, the current guidelines vary in their cut-offs for the diagnosis of AGHD between ≤3 ng/mL to ≤5.1 ng/mL for the ITT and Glucagon tests.[2-4] However, it should be noted that these limits are assay dependent and may change as newer and more sensitive assays are developed. The use of IGF-1 alone for diagnosis is only recommended for the pediatric patient with organic or genetic causes of GHD who after pediatric treatment is stopped, has very low IGF-1 values as confirmation of their lifelong GHD state. In adults with GHD, IGF may be within the normal range so a normal IGF-1 does not preclude considering the diagnosis if clinical history of head trauma, brain tumor, CNS radiation, or CNS malformations are present. Conversely, a low IGF-1 in the absence of illness, cachexia, chronic disease, or prolonged dieting is suggestive of GHD and patients should be considered for provocative testing.[2]

Precautions

This test is relatively safe and reliable if adequate precautions are observed. Patients will usually have mild symptoms of hypoglycemia during the procedure. Indeed, the absence of any symptoms suggests an inadequate test. However, severe hypoglycemic reactions can occur. Constant medical attention is required during the entire procedure. Preparations for adverse reaction should be made prior to testing and hypoglycemia reversed immediately if confusion, hypotension, loss of consciousness or seizures occur. This test is considered to be contraindicated in adults with history of symptomatic hypoglycemia or seizures.

Glucagon Stimulation Test

Purpose

Assessment of growth hormone secretion.

Rationale

See General Comments.
Glucagon has been shown to increase growth hormone release and thus has been used as a pharmacologic stimulus for growth hormone testing. It is relatively safe, although it takes longer than usual stimulation tests, and recent literature point to potential risks when used in the elderly.[5]

Procedure

- This test is usually performed after an overnight fast.
- Guidelines suggest starting an IV or a butterfly needle with or without an indwelling catheter and keep open with normal saline or a heparin lock (no glucose). Draw baseline glucose and growth hormone samples.

- Give Glucagon 0.03 mg/kg (maximum dose 1 mg), intramuscular or subcutaneous injection.
- Obtain blood samples for glucose and growth hormone at 15, 30, 60, 90, 120, 150, and 180 minutes. This timeline is longer than the other stimulation tests described because the rise in GH is variable and the peak is delayed compared to other stimuli.

Specimen Requirements

- Growth Hormone: Recommended: 1.0 mL serum for each determination. Minimum: 0.4 mL. Separate within one hour. Store and ship frozen in plastic vial.

Please note: Providing the minimum volume does not allow for repeat analysis.

Interpretation

The assessment of GH secretion capacity is complicated because of the episodic nature of GH release from the pituitary. Basal GH levels can exhibit considerable variability throughout a 24-hour period, thus limiting their clinical utility. In adult patients, response values greater than 5 ng/mL have historically been considered to reflect normal secretory function, while values below 3 ng/mL have been considered to indicate some degree of GH deficiency. The guidelines vary on the interpretation of results between >3 and <5 ng/mL.[3] Growth hormone peak values are generally seen at 120 minutes after glucagon and mean values are reported to be higher than those obtained with arginine or insulin-induced hypoglycemia. Body mass index (BMI) affects the GH response to stimuli, with increasing BMI resulting in lower values[6,7] as shown not only to insulin or arginine but also in a recent publication to glucagon.[8]

Precautions

Generally, glucagon stimulation is a safe response test as it stimulates the breakdown of glycogen in the liver to glucose and its release with a consequent rise in blood glucose. However, hypoglycemia can occur after the peak glucose rise in the first 30 to 60 minutes and therefore, serum glucose levels should be monitored closely. The test should be terminated with feeding or IV glucose if the patient becomes symptomatically neuroglycopenia or the serum glucose decreases to less than 45 mg/dL. Nausea, vomiting, and abdominal pain may also occur as a consequence of glucagon administration. A recent report suggests glucagon should be cautiously used in elderly patients with co-morbidities including vascular and cardiovascular diseases that could be potentiated by the side effects of the test, such as severe hypotension.[5]

TRH Test for Prolactin (Note: TRH is no longer available for clinical testing)

TRH Test for TSH (Note: TRH is not available at present for clinical testing)

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Additional Resources

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Pituitary Suppression Tests

Growth Hormone Suppression Test

Purpose

Acromegaly is a condition of excess growth hormone produced typically by a pituitary tumor. The uncontrolled growth hormone release may not be at a high level, but the normal suppression of growth hormone (GH) by food or glucose does not occur. The oral growth hormone suppression by glucose is the definitive test for acromegaly, however IGF-1 levels are often used as well.[1] MEN1 gene sequencing test which detects germline mutations associated with pituitary tumors may also be useful. This procedure is intended for adults.

Procedure

Patient Preparation

- The patient fasts for 10 to 16 hours, during which water may be consumed.

Test Protocol

- Take baseline blood samples:
  - Glucose: Collect in a gray top sodium fluoride tube. Label specimen as plasma. Mix well.
  - Growth hormone: Recommended: 1.0 mL serum for each determination. Minimum: 0.3 mL serum. Separate within one hour. Store and ship frozen in plastic vial.
- Give oral 75 g glucose.[2] Timing starts when patient begins drinking. Glucose should be consumed in a 5-minute period.
- Take timed blood samples for GH and glucose measurements at 30, 60, 90, 120, and 180 min.
  - Glucose: Collect in a gray top sodium fluoride tube. Label specimen as plasma. Mix well.
  - Growth hormone: Recommended: 1.0 mL serum for each determination. Minimum: 0.3 mL serum. Separate within one hour. Store and ship frozen in plastic vial.

Interpretation

In normal subjects, growth hormone is suppressed by a glucose load to below 0.4 ng/mL.[3]

Precautions

The patient should not be receiving GH-stimulating drugs. The test should not be performed on seriously ill patients and those showing metabolic response to trauma and surgery. The patient needs to be sufficiently healthy to tolerate the fast and glucose challenge.

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Thyroid Cancer Screening Tests

rhTSH Stimulation of Thyroglobulin (Tg)

Purpose

To detect residual normal thyroid tissue, persistent papillary, and follicular thyroid carcinoma.

Rationale

Thyroglobulin (Tg) has been demonstrated to be a valuable clinical marker for periodic assessment of the post-treatment status of thyroid cancer patients.[1] Historically, Tg has been measured with conventional polyclonal radioimmunoassay procedures. New immunometric methods for determining Tg have improved the value of this tumor marker by providing greater sensitivity and faster turnaround time. These new assays, however, are particularly subject to interference from Tg autoantibodies (TgAb), which occur in up to 25% of patients with thyroid cancer.[2,3] Therefore, when evaluating thyroid cancer patients, it is critical that a highly sensitive method be used to determine the presence of TgAb.[3]

Current protocols for Tg testing are offered as a comprehensive thyroglobulin testing service. All samples are first screened with a highly-sensitive TgAb assay. Samples with undetectable TgAb (<1 IU/mL) are measured with a sensitive two-site immunometric thyroglobulin (Tg-IMA) procedure. Samples containing higher amounts of TgAb are analyzed in a traditional Tg-RIA that is less influenced by TgAb.

Procedure (for adults)

- Thyrogen® (thyrotropin alfa) is manufactured by Sanofi Genzyme (see www.thyrogen.com for more information). It is supplied as a sterile white lyophilized powder for reconstitution with sterile water and intramuscular injection.
- Administer 1 mL (0.9 mg) of thyrotropin alpha intramuscularly twice – 24 hours apart.
- If radioiodine imaging is used, RAI should be given 24 hours after the second dose of thyrotropin alpha and the scan should be performed 48 hours after the RAI is given.
- Collect specimen for thyroglobulin level 72 hours after the second dose of thyrotropin alpha.

Specimen requirements

- Comprehensive Thyroglobulin: Recommended: 5.0 mL serum. Minimum: 2.0 mL serum. Separate within 1 hour. Store and ship frozen in a plastic vial.

Please note: Providing the minimum volume does not allow for repeat analysis.

Interpretation

Thyroglobulin values in patients with thyroid cancer are highly variable depending on the characteristics of the tumor, the presence of thyroid remnants or metastases, or whether samples are obtained while patients are on suppressive thyroid therapy, withdrawal, or stimulated with recombinant TSH. For patients with no thyroid remnant following thyroidectomy and radioactive iodine ablation therapy and no evidence of disease, Tg values are generally less than 3 ng/mL for Tg-RIA and less than 1 ng/mL for Tg-IMA when patients are on thyroid hormone withdrawal protocol or 72 hours following the second dose of Thyrogen®. Tg measurements may be used in conjunction with radioactive iodine thyroid scans, although a consensus conference suggests that serum Tg measurements after Thyrogen® stimulation or thyroid hormone withdrawal are more sensitive than scans in patients with papillary thyroid carcinoma or low-risk follicular carcinoma.[3] A value for Tg-IMA of 2 ng/mL following Thyrogen® stimulation or thyroxine withdrawal often indicates residual thyroid tissue or persistent thyroid carcinoma.

Calcitonin General Comments

Calcitonin is a 32 amino acid peptide secreted by C-cells of the thyroid. Measurement of calcitonin has been found to be of value in detecting C-cell hyperplasia and subsequent medullary carcinoma of the thyroid (MTC).[2] Elevated calcitonin levels are not specific for medullary carcinoma and may be found in renal failure, pregnancy, sub-acute thyroiditis, pernicious anemia, cystic fibrosis, various bone diseases, a variety of tumors (particularly oat cell carcinoma of the lung), and infants in the first year of life.[2] Medullary thyroid cancer (MTC) occurs as a sporadic disease in approximately 70% of cases (1% -7% have *RET* mutations)[7] and familial MTC accounts for the remaining 30%, with a very high percentage of *RET* mutations.[4]

The *RET* proto-oncogene on chromosome 10 encodes a receptor protein tyrosine kinase. Germline mutations in this gene have been shown to lead to familial MTC development in cells derived from neural crest tissue (C-cells) as well as hyperplasia or tumor formation in the adrenal medulla and parathyroid glands (MEN2A).[4] MEN2B patients have familial or sporadic MTC with additional features (ganglioneuromas of the digestive tract, skeletal abnormalities).[5] Calcitonin levels are elevated early in the development of MTC when premalignant C-cell hyperplasia is present. Although the majority of patients with this tumor have clearly elevated calcitonin levels, a significant number of patients with *RET* mutations have borderline or normal calcitonin levels.[6] In the past, stimulation tests with pentagastrin and calcium have been useful in detecting early abnormalities of calcitonin secretion, often before clinical evidence of the tumor is present. Further, because medullary carcinoma of the thyroid commonly occurs in families and has an autosomal dominant mode of inheritance with variable penetrance, provocative tests with pentagastrin and calcium have been useful in the past for screening family members. However, studies suggest that molecular genetic analysis of the *RET* proto-oncogene is superior to biochemical screening and should be performed at an early age, before 1

year of age for children in families with *RET* mutations.[6] It should be emphasized that when a familial form of MTC is suspected, it is suggested that the index case should be screened for a germline *RET* mutation with analysis of exons 10, 11, and 13-16, and if they are negative, the remaining 15 *RET* exons should be examined.[7] At-risk family members can then be screened for the identified *RET* mutation. If no *RET* mutation is found, a small risk of 0.18% exists for hereditary MTC in first-degree relatives.[7] If clinically indicated, provocative calcitonin screening can be considered in these patients.[7] However, at present, pentagastrin is not commercially available. Hence, calcium infusion can be used for screening since calcium has been shown to augment calcitonin secretion.[2] Basal and calcium infusion-stimulated calcitonin should not be used in place of *RET* molecular screening.[7]

Calcium Infusion Tests

Purpose

The detection of medullary thyroid carcinoma.

Rationale

Calcium has been shown to increase calcitonin levels above the normal range in patients with C-cell hyperplasia or early medullary carcinoma who have normal baseline calcitonin levels. The calcitonin response can also be used post-operatively to detect residual medullary thyroid carcinoma or recurrence of the tumor. This procedure is intended for adults or children.

Procedure

- The patient is NPO overnight (shorter time in young children).
- Start IV with normal saline and draw baseline calcitonin level.
- With the patient supine, be sure IV is securely in place in a large peripheral vein and that it flushes well, since extravasation of calcium can result in a severe local reaction and necrosis.
- Infuse 2 mg/kg of elemental calcium as calcium gluconate (10% calcium gluconate = 100 mg/mL = 9 mg elemental calcium/mL). Dilute 10% calcium gluconate to 2% solution (1 part calcium gluconate to 4 parts normal saline) and infuse carefully over 60 seconds. 2 mg of elemental calcium/kg can be given as calcium chloride (27% elemental calcium) or calcium acetate (13% elemental calcium).
- Monitor blood pressure and pulse during infusion.
- Draw blood samples for calcitonin at 0, 1, 2, 5, 10, and 30 minutes.

Specimen Requirements

- Calcitonin: Recommended: 1.0 mL serum for each determination. Minimum: 0.5 mL serum. Separate within 1 hour. Store and ship frozen in a plastic vial.

Please note: Providing the minimum volume does not allow for repeat analysis.

Interpretation

Calcitonin measurements are method and laboratory dependent. Thus, the results must be interpreted with this caveat in mind. Normal calcitonin levels in adults are less than 10 pg/mL using two-site immunometric assays. Calcitonin levels are lower in females than in males.[8] Currently, there is no published data for calcium-stimulated calcitonin levels in children.

No data using two-site assays have been published.

Normal adults demonstrated a two-fold to five-fold increase in calcitonin when measured by RIA.[9] Gender-related differences were not examined.

Precautions

Infiltration of calcium into subcutaneous tissues will cause a severe local reaction and may result in necrosis at the site. Calcium infusion may cause flushing, a feeling of warmth, an urge to urinate, and a sensation of gastric fullness lasting one to five minutes.

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Gonadal Stimulation Tests

HCG Stimulation Test

Purpose

Assessment of gonadal Leydig cell responsiveness in males with suspected Leydig cell agenesis or hypoplasia; differentiation of anorchia and undescended testes; diagnosis of 5 alpha-reductase-2 deficiency and biosynthetic errors of testosterone synthesis. Basal anti-müllerian hormone (AMH) and Inhibin B levels are accurate discriminators of Sertoli cell integrity. They can be used to discriminate between anorchia, dysgenetic testes, and functional but undescended testes.[1]

Rationale

Human chorionic gonadotropin (HCG) is a glycoprotein that possesses biologic and immunologic similarities to luteinizing hormone (LH). Like LH, it can stimulate Leydig cell function in males and increase testosterone production. Low testosterone output resulting from testicular failure usually leads to increased LH release. In comparison, FSH stimulates the Sertoli cells, which secrete inhibin B.[2] Thus, FSH and inhibin B may be better testing options to assess Leydig cell function than LH. In addition, the decline in AMH concentrations is inversely related to intratesticular testosterone and can therefore demonstrate Sertoli cell function and androgen activity in the testes.[2] Moreover, in prepubertal children between the ages of three and nine years, “central neural inhibition” of gonadotropin secretion can mask the lack of gonadal feedback on gonadotropin secretion so that even girls with gonadal dysgenesis and boys with anorchia may have normal LH and FSH levels at these ages. Consequently, a dynamic stimulation test is required to elucidate suspected gonadal deficiency. In the normal male, the testes respond to HCG with a pronounced increase in synthesis and release of testosterone. However, in females, ovarian steroid synthesis is primarily FSH-dependent and hence this test is not employed in them.

Several HCG stimulation test protocols are available. Generally, the testosterone response to HCG increases in proportion to the daily dose and to the number of injections. The protocol outlined below employing three HCG injections was chosen because of its relative simplicity and the availability of reliable data on age-related normal responses. Detailed normal response data have also been established for prolonged tests consisting of seven alternate-day injections of HCG.[3] This procedure is intended for infants and children.

Procedure

- Draw a baseline blood sample for Testosterone, FSH, LH, and where appropriate, Dihydrotestosterone and intermediates.
- Inject 1500 IU HCG intramuscularly every other morning on days 1, 3 and 5. Alternatively, HCG, 5000 IU/m2 can be injected intramuscularly in a single dose.
- Twenty-four hours after the third injection (day 6), or 72 hours after the single injection, draw a blood sample for testosterone and DHT and intermediates if desired.

Specimen Requirements

- Testosterone: Recommended: 0.8 mL serum for each determination. Minimum: 0.4 mL serum. Separate within one hour. Store and ship frozen in plastic vial.

Please note: Providing the minimum volume does not allow for repeat analysis.

Interpretation

Normal males respond to HCG administration with a significant rise in serum testosterone levels. However, that response is age-dependent. Infants younger than six months of age may exhibit relatively high baseline levels of testosterone with a brisk response to HCG. Later on during the first year of life, both baseline and HCG stimulated testosterone levels decline and increase again only during puberty. A table of age-related expected values is given below. Testosterone and DHT responses were obtained after administration of three 1500 IU doses of HCG (no normative data for intermediates with the one and three dose HCG test are available).

A normal response to HCG may be seen in patients with unilateral or bilateral undescended testes, while lack of response is compatible with anorchia or Leydig cell agenesis. A reduced response is generally observed in Leydig cell hypoplasia or other causes of primary hypogonadism.

The HCG test is also helpful in the differential diagnosis of male pseudohermaphroditism when 5 alpha-reductase-2 deficiency and abnormalities in testosterone synthesis are suspected. Patients with 5 alpha-reductase-2 deficiency exhibit impaired conversion of testosterone to dihydrotestosterone, but the basal unstimulated testosterone/DHT ratio is frequently not high enough to unequivocally establish the diagnosis. However, following HCG administration, testosterone increases in both normal boys and those with 5 alpha-reductase-2 deficiency, whereas dihydrotestosterone rises substantially only in normals. In normal prepubertal males, the mean T/DHT ratio following HCG stimulation is 10.7 with ranges from 3.5-14. In male infants, the stimulated ratio is somewhat lower, and is usually less than 10. The T/DHT ratio in prepubertal patients with 5 alpha-reductase-2 deficiency generally exceeds 30. In adults, the discriminatory value of the post HCG T/DHT ratio is even higher. Normal patients respond with ratios from 8-16, while patients with 5 alpha-reductase-2 deficiency exhibit T/DHT ratios from 35-84.

Testosterone and DHT Response to 3X1500 Iu Doses of HCG

		BASELINE			POST-HCG STIMULATION		
Normal Subjects (Males)		**Testosterone (ng/dL)**	**DHT (ng/dL)**	**T:DHT Ratio**	**Testosterone (ng/dL)**	**DHT (ng/dL)**	**T:DHT Ratio**
Infants 1 wk - 6 mos.	Mean	190	43	4.4	395	76	7.2
(N = 16)	Range	4 - 530	<2 - 60	2 - 8	180 - 735	13 - 183	1.5 - 15.5
Prepubertal 1 - 8 yrs	Mean	4.6	<3	ND	150	14	10.7
(N = 11)	Range	<2 - 12	<3	ND	83 - 370	17 - 36	3.5 - 14
Stage 1	Mean	24	3.7	ND	196	20	10.0
(N = 7)	Range	5 - 60	<2 - 10	ND	75 - 600	8 - 54	4.1 - 11.6
Stage 2 (puberty)	Mean	53	8.1	6.5	272	22	12.1
(N = 8)	Range	19 - 195	4 - 19	3 - 15	140 - 1030	19 - 75	3.9 - 14.6
Stage 3 (puberty)	Mean	208	19	10.9	710	61	11.6
(N = 13)	Range	82 - 400	11 - 36	6 - 17	460 - 1505	21 - 109	5.7 - 13.8
Stage 4 (puberty)	Mean	415	36	11.5	1270	98	12.9
(N = 13)	Range	130 - 550	20 - 56	7 - 14	570 - 1950	56 - 169	7.0 - 15.3
Stage 5 (puberty)	Mean	531	48	11.1	1285	105	12.2
(N = 10)	Range	120 - 805	29 - 67	7 - 16	980 - 2200	78 - 237	7.4 - 16.1

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Polyuria/Polydipsia Tests

Water Deprivation Test

General Comments

The pathologic causes of the polyuria-polydipsia syndrome (PPS) are diverse,[1] comprising many forms of central diabetes insipidus (DI), whether partial or complete; nephrogenic; primary polydipsia leading to increased water intake; or physiologic as in pregnancy with increased placental production of vasopressinase.[2] An increasing number of genetic entities that cause PPS are being discovered.[3,4] A step in the diagnosis of many of these conditions involves a well-controlled and closely-monitored water deprivation test to quantitate a patient's CNS/posterior pituitary, physiologic and renal response to a period of no water intake.[2]

Purpose

Evaluation of patients suspected of having diabetes insipidus.

Rationale

Water balance and plasma osmolality are tightly controlled in humans by thirst, anti-diuretic hormone (ADH) secretion from the posterior pituitary, and the kidneys. A small change, either upward or downward, in plasma osmolality elicits either increased or decreased release of ADH from the posterior pituitary gland with resultant antidiuresis or diuresis at the renal level. ADH acts on the kidney tubules by binding to the V2 receptors affecting the aquaporins, which mediate the transport of free water from the nephrons into the circulation. Maximum antidiuresis occurs at plasma ADH concentrations of 2-4 pg/mL. Patients with diabetes insipidus (either central or nephrogenic) have polyuria and urinate large volumes (>2 liters/m2/24 hr in older children and adults) of dilute urine. Before undertaking the water deprivation test to determine the patient's ability to concentrate their urine and respond to desmopressin (DDAVP), it is prudent to exclude osmotic and metabolic causes of polyuria, as well as to document the 24-hour volume of urine. Serum electrolytes, glucose, calcium, creatinine, plasma osmolality, and simultaneous first morning urine osmolality should be obtained. A diagnosis of diabetes insipidus can sometimes be made simply from the first AM urine osmolality and concurrent plasma osmolality.[5] This procedure is intended for adults or children.

Procedure

- Water deprivation tests should never be conducted at home. They are performed in the hospital or outpatient setting under very close medical supervision during the day. Patients must be observed closely and prevented from receiving free access to water.
- The patient may be allowed fluids overnight, but no solid breakfast.
- NPO at 8:00 AM. Record weight and vital signs. Draw blood for electrolytes, plasma osmolality, and plasma ADH level. Collect urine for urinalysis, specific gravity, and urine osmolality.
- Collect all urine specimens for osmolality. Draw serum electrolytes, creatinine, and plasma osmolality at two hours.
- Weigh patient and record vital signs at two hours.
- Stop test before 7 to 8 hours if weight decreases >3% to 5% from baseline, plasma osmolality exceeds 300 mOsm/kg or the patient becomes hypotensive and/or tachycardic.
- At the end of 7 to 8 hours of water deprivation, collect urine for specific gravity, osmolality and plasma for electrolytes, osmolality, and ADH level. For additional details on the interpretation of results for pediatric patients, please refer to Figure 11-12 on page 418 in the Pediatric Endocrinology textbook.[5] For additional details on the interpretation of results for adult patients, please refer to Table 1 in de Fost M et al.[6] Note: In some patients who have not lost 3% to 5% of body weight and have normal vital signs, and appear to be well-hydrated, the test sometimes has been prolonged until urine osmolality becomes fixed; that is, the osmolality does not change more than 10% between consecutive voids. A further increase in urine osmolality after desmopressin administration in the patient suggests central diabetes insipidus, while no change indicates maximal concentration ability has been achieved and the patient may have nephrogenic diabetes insipidus or primary polydipsia with loss on the counter current concentrating mechanism. The plasma ADH level achieved before the desmopressin is given may clarify the diagnosis.

- Continue to monitor input and output, urine volume, specific gravity, and osmolality with each void. Restrict patient's intake of fluids to the amount of urine excreted during the test plus that voided until the antidiuretic effect of the desmopressin injection has waned (i.e., urine-specific gravity 1.005 or lower, urine osmolality less than 200). Solids may be eaten ad lib. (Caveat: Solids contain water.)
- At 8:00 AM the next morning (after the desmopressin injection), collect blood for electrolytes, creatinine, and plasma osmolality as well as urine for osmolality.

Specimen Requirements

- ADH: Recommended: 2.0 mL EDTA plasma only. Centrifuge in a refrigerated centrifuge, separate plasma, and **freeze immediately.** Transfer specimen to a plastic transport tube before freezing.

Interpretation

The interpretation of the water deprivation test is complicated by several factors which include 1) the fact that polyuria may compromise the ability of the kidney to respond to ADH, 2) cortisol deficiency may mask central diabetes insipidus, and 3) the criteria for a response to 7 to 8 hours of water deprivation in children differs widely depending on the report cited. To aid in the interpretation of results for pediatric patients, please refer to Figure 11-12 on page 418 in the Pediatric Endocrinology textbook.[5] To aid in the interpretation of results for adult patients, please refer to Table 1 in de Fost M et al.[6] Patients with nephrogenic diabetes insipidus generally do not concentrate their urine to greater than 300 mOsm/kg after water deprivation, do not respond to multiple physiologic doses of desmopressin over several days, and have elevated ADH levels. Furthermore, patients with central diabetes insipidus can sometimes concentrate adequately on one day, however, be unable to concentrate when deprived of water on a subsequent day. Although rarely performed, it has been suggested that in children older than five years of age, a definitive diagnosis of central versus nephrogenic versus dipsogenic diabetes insipidus can be made utilizing hypertonic saline infusion and the measurement of plasma ADH levels.[7,8]

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Hyperinsulinemic Hypoglycemia Tests

General Comments

Hyperinsulinism is the most common cause of hypoglycemia in infants and children after the first day of life.[3] Recent studies have defined the pathophysiology of basic genetic forms of persistent hypoglycemia due to hyperinsulinism. These include mutations in the sulfonylurea receptor, the inward-rectifying 6.2 kDA potassium channel, glucokinase and glutamate dehydrogenase gene.[1,2] Patients with mutations in the glutamate dehydrogenase gene are leucine-sensitive and have elevated ammonia levels.[2,3] Hyperinsulinism should be suspected in a child who develops hypoglycemia a few hours after a meal and it not acidotic (increased lactate) or ketotic (increased beta-hydroxybutyrate or aceto-acetate levels). Mid-line defects such as cleft lip and/or palate, optic hypolasia and micropenis are seen in patients with hypopituitarism. Critical for the diagnosis of any form of hyperinsulinism is the demonstration of a measurable insulin level greater than 2 µU/mL in the face of hypoglycemia (a serum glucose below 50 mg/dL). In addition, hyperinsulinism is associated with low ketone bodies and free fatty acids at the time of hypoglycemia as well as a glycemic response of greater than 30 mg/dL to glucagon stimulation when hypoglycemic. Patients with hyperinsulinism usually require greater than normal amounts of glucose (5-10 mg/kg min. in the neonate) to maintain their blood sugars in the normal range. At the time of hypoglycemia it is essential to obtain a critical sample of blood for intermediate metabolites and hormones in order to clarify the etiology of the hypoglycemia. However, rarely are the appropriate tests done at the time of the first spontaneous hypoglycemic

episode. Therefore, it may be incumbent upon the physician to do a careful, closely-observed diagnostic fast in order to obtain these samples. The diagnostic fast to obtain critical samples lasts as long as necessary to achieve hypoglycemia (serum glucose less than 50 mg/dL) and may in adults entail a 48- to 72-hour fast. Since there are questions about the safety of diagnostic fasts in patients with B-oxidation defects, total and free carnitine and acetylcarnitine profile levels should be ascertained in any child with nonketotic hypoglycemic episodes or with a suspected fatty acid oxidation defect before a fast is attempted.

Diagnostic Fast[1-4]

Procedure

- NPO: In infants and children, it is necessary to have an IV access site available in case symptomatic hypoglycemia occurs.
- Monitor glucose every 30 to 120 minutes depending on age, starting serum glucose and clinical history until glucose decreases to less than 50 mg/dL.
- At the time of hypoglycemia, draw critical sample for glucose, lactate, pyruvate, beta-hydroxybutyrate, free fatty acids, ammonia, insulin, C peptide, cortisol and growth hormone.
- Obtain urine for ketones, reducing substances, amino and organic acids.
- Thereafter give glucagon: neonates 0.3 mg/kg, children 0.1 mg/kg (maximum dose 1 mg) IV Obtain serum glucose at 10, 20 and 30 minutes.
- In the event of symptomatic hypoglycemia (i.e., a seizure), administer 2 mL/kg 10% glucose (0.2 g/kg) of glucose IV over one minute and then 5 mL/kg/hr (8 mg/kg/min) of 10% glucose or more IV as needed to maintain the glucose in the normal range.

Specimen Requirements

- Insulin: Recommended: 1.0 mL serum for each determination. Minimum: 0.5 mL serum. Separate within one hour. Store and ship frozen in a plastic vial.
- Cortisol: Recommended: 0.5 mL serum for each determination. Minimum: 0.1 mL. Separate within one hour. Store and ship frozen in a plastic vial.
- Growth Hormone: Recommended: 1.0 mL serum for each determination. Minimum: 0.4 mL. Separate within one hour. Store and ship frozen in plastic vial.
- C-Peptide: Recommended: 1.0 mL serum for each determination. Minimum: 0.5 mL. Separate within one hour. Store and ship frozen in plastic vial.

Please note: Providing the minimum volume does not allow for repeat analysis.

Interpretation

The criteria for diagnosing hyperinsulinism using the critical sample when the glucose is less than 50 mg/dL is 1) an insulin level greater than 2 µU/mL, 2) decreased free fatty acids <1.5 mmol/L, 3) decreased ketone bodies, betahydroxybutyrate <2.0 mmol/L and an inappropriate rise in serum glucose after glucagon (>30 mg/dL).[1-3] In addition, glucose infusion rates greater than normal (above 8 mg/kg/min. in infants) to maintain normoglycemia and a low IGFBP-1 level are supportive of the diagnosis.[1,3] Hypoglycemia due to hyperinsulinism in the adolescent is usually due to an insulinoma.[3] A more prolonged fast may be necessary in the adolescent or adult to rule out hyperinsulinism.[2,4]

Calcium Infusion Test[5,6]

Purpose

A rise of intracellular calcium concentration can mediate a release of insulin, proinsulin and C-peptide.

Specimen Requirements

- Calcium: Recommended: 1.0 mL serum for each determination. Minimum: 0.5 mL serum. Separate within one hour. Store and ship frozen in a plastic vial.
- Glucose: Recommended: 0.5 mL serum for each determination. Minimum: 0.1 mL. Collect in a tube with NaF. Separate within one hour. Store and ship frozen in a plastic vial.
- Insulin: Recommended: 1.0 mL serum for each determination. Minimum: 0.5 mL serum. Separate within one hour. Store and ship frozen in a plastic vial.

Please note: Providing the minimum volume does not allow for repeat analysis.

Interpretation

Adult Patients

Following a calcium gluconate infusion (10 mg Ca++/kg) for more than two hours, a marked rise in insulin, a concomitant hypoglycemia (necessitating testing in an environment able to perform glucose levels quickly and administer IV glucose if necessary) has been noted in adults with insulinomas.[7]

Pediatric Patients

Causes of persistent neonatal and infant hypoglycemia are well outlined as an aide to diagnosis of various congenital, monogenetic forms of hypoglycemia (such as sulfonourea receptor (SUR-1) or its associated potassium channel (KIR6.2) defects).[2] An infant-tailored calcium infusion protocol has been proposed administering 0.1 mEq calcium/kg body weight over 60 seconds.[5] Precautions should be noted appropriately. In part, this procedure suggests that plasma glucose, serum calcium, and plasma insulin samples should be obtained from a contralateral vein at -600 second, -300, 0, +60, +180, +300, and +600 seconds relative to the start of the calcium infusions. Medications should be discontinued before the studies are begun. In children with unstable hypoglycemia, intravenous dextrose may be infused continuously as needed to maintain plasma glucose levels between 3.3 to 5.0 mmol/L (60 to 90 mg/L) before and throughout the study. The CaAIR should be calculated as the change in insulin levels between the mean of the baseline values and the mean of the acutely stimulated values at +60 and +180 seconds. The rise in serum calcium should be calculated in the same manner.

Precautions

Infiltration of calcium into subcutaneous tissue will cause a severe reaction and may result in necrosis at the site. Calcium infusion may cause flushing, a feeling of warmth, an urge to urinate, and a sensation of gastric fullness lasting one to five minutes.

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Insulin Resistance Tests

Oral Glucose Tolerance Test (OGTT)

Purpose

Diagnosis of impaired glucose tolerance (IGT).

Rationale

Following ingestion of a glucose load, blood glucose rises, leading to a prompt increase in circulating insulin in normal subjects. This, in turn, enhances glucose entry into cells, which promotes glycolytic metabolism and limits the extent and duration of hyperglycemia. In diabetic patients, however, the insulin response may be delayed, insufficient or completely blunted, allowing blood glucose to rise above, or stay above, the normal range. In type 1 diabetes where clinical symptoms are usually manifest, there is little indication for performing an OGTT. However, when non-overt diabetes is suspected according to the following criteria, OGTT is often indicated. The American Diabetes Association (ADA) diagnostic criteria for considering a diagnosis of diabetes are: a Hemoglobin A_{1c} ≥6.5%, a fasting blood glucose ≥126 mg/dL (7.0 nmol/L), a two-hour post-prandial blood sugar ≥200 mg/dL (11.1 nmol/L), or a random plasma glucose ≥200 mg/dL (11.1 nmol/L) .[1] The World Health Organization criteria are identical regarding fasting blood glucose (≥126 mg/dL) and for a two-hour post-prandial blood sugar (≥200 mg/dL).[2] In patients with obesity or other factors predisposing to type 2 diabetes, an OGTT with concomitant measurement of plasma insulin may help identify those patients with secretory abnormalities of insulin, as well as those with resistance and covert type 2 diabetes. The oral glucose tolerance test is the most sensitive method of detecting early diabetes. A study reported that impaired glucose tolerance was detected in 25% of 55 obese children and 21% of 112 obese adolescents.[3] In addition, silent diabetes was diagnosed in four adolescents (4%). Studies suggest that insulin resistance is a transitional phase in the development of type 2 diabetes. There is rarely an indication in childhood to do an OGTT to rule out hypoglycemia.[2]

Procedure

- The test should be delayed for at least two weeks following any acute illness.
- Many drugs are known to interfere with either the laboratory test for serum glucose, or are associated with impaired glucose tolerance and should thus be avoided whenever possible.
- Physical activity should be encouraged in the days preceding the test.
- Children should be maintained on a diet in which 50% of the calories are consumed as carbohydrates, or a minimum of 150 grams of carbohydrates daily. Quiet activity is permitted during the test.
- Following an overnight or 12-hour fast, a baseline blood sample for glucose, insulin, proinsulin, and a lipid profile is obtained. Glucose, 1.75 gm/kg of ideal body weight up to 75 grams, is given orally over no more than five minutes.
- Blood samples for glucose and insulin are collected at 15, 30, 60, 90, 120, 150, 180, 240, and 300 minutes.

Specimen Requirements

- Glucose: Recommended: 0.5 mL serum for each determination. Minimum: 0.1 mL. Collect in a tube with sodium flouride (NaF). Separate within one hour. Store and ship frozen in a plastic vial.
- Insulin: Recommended: 1.0 mL serum for each determination. Minimum: 0.5 mL serum. Separate within one hour. Store and ship frozen in a plastic vial.
- Proinsulin: Recommended: 1.0 mL EDTA plasma only for each determination. Minimum: 0.5 mL plasma. Separate within one hour. Store and ship frozen in a plastic vial.

Please note: Providing the minimum volume does not allow for repeat analysis.

Interpretation

Diabetes Mellitus in Children

Either of the following is considered diagnostic of diabetes:

- Presence of the classic symptoms, such as polyuria, polydipsia, ketonuria, and rapid weight loss, with a random plasma glucose ≥200 mg/dL (11.1 nmol/mL), Hemoglobin A_{1c} ≥6.5% or diabetic retinopathy. A glucose tolerance test is not indicated in these individuals.[2]
- In asymptomatic individuals, an elevated fasting glucose concentration ≥126 mg/dL (7.0 nmol/mL) and/or a sustained elevated glucose level during the OGTT with a glucose ≥200 mg/dL at two hours.[1]

Impaired Glucose Tolerance (IGT) in Children (Insulin Resistance)

Either of the following is considered for increased risk of diabetes[1]:

- The fasting glucose concentration must be below the value that is diagnostic of diabetes (126 mg/dL).
- The glucose concentration two hours after an oral glucose challenge must be elevated (140-199 mg/dL).

The insulin response to OGTT is somewhat age-dependent. Obese children display an enhanced response consistent with impaired glucose tolerance, which plays a role in their long-term prognosis with regard to the evolution of diabetes mellitus.[3] Children with type 2 diabetes, at least initially, have a delayed, though often enhanced insulin response to an oral glucose load. The simplest method of determining insulin resistance (although not as sensitive as the OGTT) is the determination of the fasting glucose/insulin ratio or simply the fasting insulin level. A glucose/insulin ratio of less than seven connotes some degree of insulin resistance.[4] Other indexes of insulin resistance such as the HOMA-IR or QUICKI can be calculated from the fasting glucose/insulin ratio.[5]

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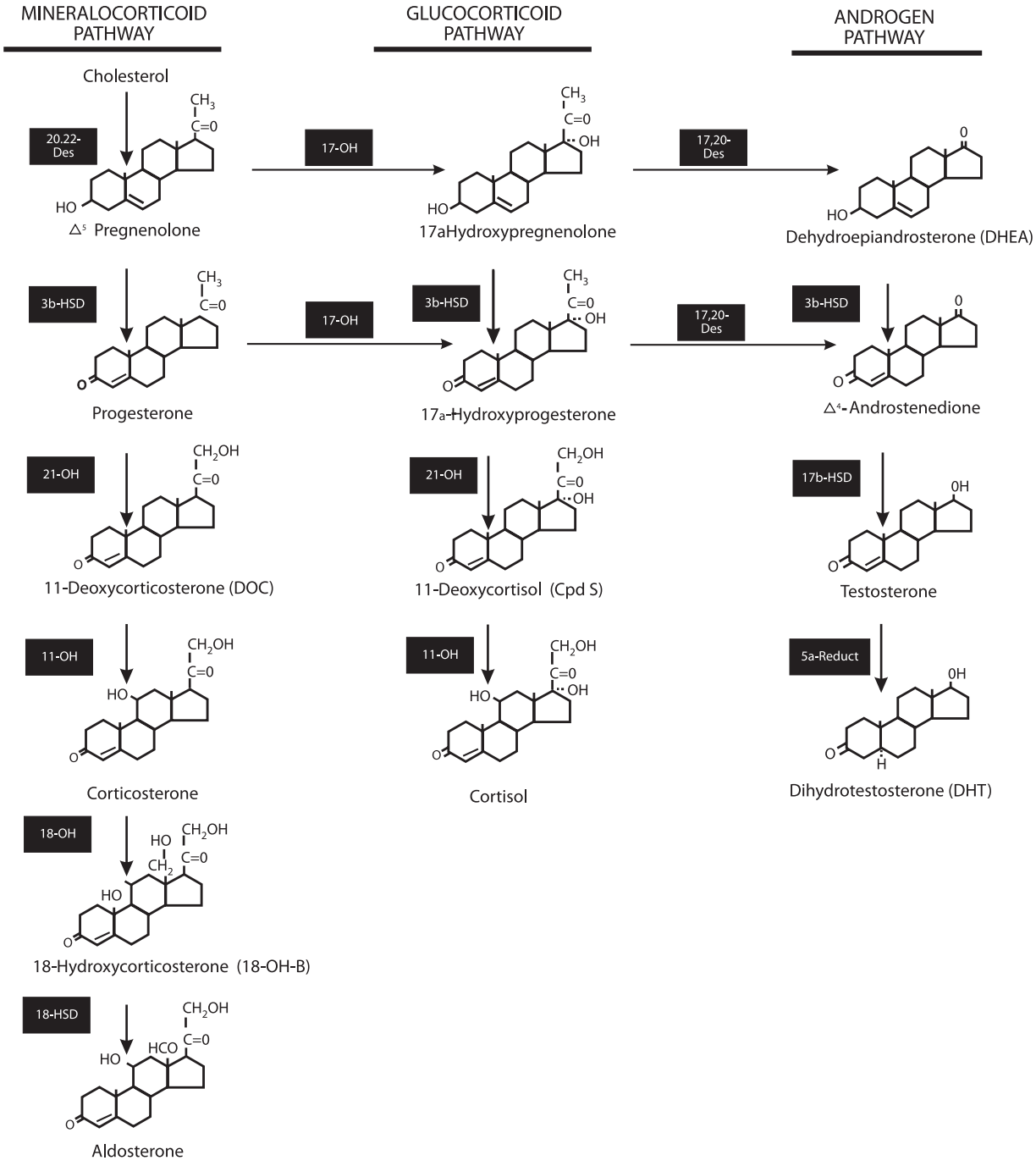
Charts and Data Tables

Adrenal Steroid Response To ACTH



Pediatric Steroid Profiles for the evaluation of defects in steroid biosynthesis

Labcorp Test N°	Profile Name	Profile Components
502682	5-Alpha-Reductase Profile	DHT, Testosterone
501560	Adrenal Androgen Profile	DHEA-Sulfate, Estradiol, Estrone, Progesterone, SHBG, Free and Total Testosterone
501568	CAH Profile 1 (21-OH Deficiency)	Androstenedione, Cortisol, DHEA, 17-OH Progesterone, Testosterone
500176	CAH Profile 2 (11-OH Deficiency)	Androstenedione, 11-Desoxycortisol (Specific S), Cortisol, DHEA, 17-OH Progesterone, Testosterone
502350	CAH Profile 3 (17-OH Deficiency)	Corticosterone, Cortisol, DHEA, 17-OH Progesterone, Progesterone
501023	CAH Profile 4 (3bHSD Deficiency)	Androstenedione, Cortisol, DHEA, 17-OH Pregnenolone, 17-OH Progesterone
502348	CAH Profile 5 (17,20 Des. Def.)	Androstenedione, Cortisol, DHEA, 17-OH Pregnenolone, Progesterone, 17-OH Progesterone, Testosterone
500175	CAH Profile 6 (Comprehensive)	Androstenedione, 11-Desoxycortisol (Specific S), Cortisol, DHEA, DOC, 17-OH Pregnenolone, Progesterone, 17-OH Progesterone, Testosterone
500166	CAH Profile 7 (Treatment Profile)	Androstenedione, 17-OH Progesterone, Testosterone
500285	Mineralocorticoid Profile	Aldosterone, 18-hydroxycorticosterone, Corticosterone, DOC
500767	Premature Adrenarche Profile 1	Androstenedione, DHEA-Sulfate, 17-OH Progesterone, Testosterone
500913	Premature Adrenarche Profile 2	Androstenedione, DHEA, 17-OH Pregnenolone, 17-OH Progesterone, Testosterone

Adrenal Steroid Response To ACTH: Pediatrics

Introduction

The data presented in this booklet summarize the normal studies performed at Labcorp's Endocrinology Center of Excellence laboratory over the past several years on adrenal steroid response to ACTH in children. Through collaborative efforts with several pediatric institutions, we have been able to establish normal basal and response values for nearly all age groups. These comprehensive data now extend from premature infants through infancy, early childhood, pubertal ages, and adults. To our knowledge, they are the most extensive data currently reported on ACTH stimulation in children. Since the ACTH response of many adrenal steroids varies dramatically with age, the availability of these age-related normal data has proven to be very useful in the interpretation of laboratory results and the evaluation of adrenal function in pediatrics.

Test Protocol and Conditions

Stimulation tests were performed on ambulatory subjects (when applicable) using a standard ACTH dose of 25 units (0.25 mg Cortrosyn®) given as an IV bolus. Blood samples were drawn at 0 and 60 minutes. No attempt was made to control posture in older subjects, or dietary intake of electrolytes. In some studies involving older children and adults, additional samples were drawn at 30 minutes after ACTH. These data have been omitted from the table because of space limitations, and also because our results do not support the need for additional poststimulation values. The amount of ACTH used for the short 60-minute test does not appear to be important, since all commonly used doses are pharmacological. Comparable response values have been observed with doses ranging from 0.05 - 0.5 U/kg.

Data Tables

Data are presented as the range and mean for baseline and 60-minute samples and for the increase above baseline or delta (—) value. Precursor:product ratios were determined separately for each subject and are not computed from the means and ranges of the respective steroids. The number of control subjects is given in the discussion section for each age group.

Assay Procedures

The steroids in these studies were determined with research quality procedures that have been used in the laboratory at Labcorp's Endocrinology Center of Excellence laboratory for many years. All of the testing is conducted using HPLC/MS-MS methodologies, and most assays include additional extraction protocols to ensure consistent results. Steroid-free samples (blanks), known standards, multiple-level control pools, and randomly repeated samples are included in every assay run. An involved quality control system serves to prevent assay blanks by insuring that nitrogen drying systems are free of organic material, that solvents are redistilled, and that all glassware, chromatography media, and other materials used in the assays are solvent-washed to eliminate possible interference from nonvolatile organic residues. This program is costly and time-consuming, but it is essential for reliable pediatric endocrine testing on a continuous daily basis.

Assays are performed in the laboratory by well-trained graduate-level biochemists and technologists who have demonstrated the ability to handle this kind of methodology reliably. The laboratory is supervised by PhD-level chemists who have been involved with the development of steroid assays and laboratory management for many years. Assays are reviewed by these section supervisors before any results are reported.

The reliability of our routine steroid methods has been examined during development and in numerous studies over the years. These evaluation procedures have included combinations or, in some cases, all of the following verification checks:

1. Evaluation of dose-response linearity obtained with multiple volumes of sample extracts and/or chromatography eluates.
2. Comparison of the values obtained with alternate chromatography procedures.
3. Comparison of values after a second or additional chromatography procedure is added.
4. Evaluation of results in abnormal sera known to contain high levels of precursor steroids.
5. Evaluation of the steroid response to physiological maneuvers, primarily stimulation and suppression tests.
6. Comparison of normal and abnormal values with those obtained using established assays.
7. Use of special chemical alterations or derivatives in situations where assay specificity requires additional confirmation.

Interpretation of Results

Some caution is indicated in the interpretation of ACTH response tests in children. In most cases, laboratory results are clear because they either fall within the normal reference interval or stimulate well beyond the upper limit of normal in those patients with biosynthetic defects. In other situations, however, the interpretation of laboratory data is not apparent, and a number of variables, including those outlined below, should be considered.

1. **Age-Related Changes —** The ACTH response of many clinically relevant steroids changes substantially with age; therefore, meaningful interpretation of results requires valid normal data for the age group in question.
2. **Newborns and Premature Infants —** It is recommended that newborn samples be taken on day two or three of life when steroid levels have become more stabilized and normal reference data are available. Values of many steroids in premature infants including 17-OH-progesterone are much higher than in full-term infants. Other steroids, in addition to 17-OH-progesterone, should be ordered to evaluate possible defects in these cases.
3. **Methodology —** The steroid values obtained in various laboratories are often not comparable because of methodological differences. Comparing results from one laboratory with either normal reference intervals or results from another laboratory may be misleading. In many situations, it is important to have normal data that are specific for the methods being used in the laboratory performing the test.
4. **Variability of ACTH Response in Children —** The results from large numbers of ACTH response tests processed in our laboratory over the past few years clearly demonstrate that a large variation exists in the response patterns of different steroids in children. Unusual response patterns are sometimes seen which are unlikely to be associated with any abnormality in steroid biosynthesis.
5. **Use of Statistical Data —** The clinical evaluation of test results based on strict application of the statistical data published by Labcorp or other laboratories is questionable because of the number of factors that can influence test results. While the ACTH response test has been useful for amplifying defects in steroid biosynthesis and identifying cases of late-onset congenital adrenal hyperplasia (CAH), care should be taken to avoid interpreting data too narrowly. It should be recognized that defects capable of causing clinical problems are usually associated with dramatic elevations in precursor steroids and usually become obvious after stimulation with ACTH.
6. **Variability of Steroid Responsiveness to ACTH —** The ACTH responses of different adrenal steroids vary substantially and are frequently not well correlated. This situation results in wide variability in both the absolute levels and the ratios of different steroids. In addition, those steroids that are very sensitive to ACTH, such as 17-OH-pregnenolone and corticosterone, may have more pronounced fluctuations during the day. Unstimulated values may change substantially. This kind of variation must be considered when evaluating laboratory data.
7. **Precursor/Product Ratios —** Precursor:product ratios are useful, but they should not be rigidly interpreted and must be evaluated relative to the actual stimulated levels of the steroids involved. In baseline samples, the use of precursor:product ratios is not meaningful unless the steroid levels are highly elevated.
8. **Increases Above Baseline —** Evaluating results by computing the response as a percentage or multiple increase above baseline is also

useful, but again must be interpreted relative to the actual stimulated levels of the steroids in question. The serum levels in baseline samples can vary substantially as a result of diurnal variation, episodic secretion, stress, or other conditions. Thus, the percentage increase can vary significantly depending on the time of day and conditions under which the test was performed. For example, if response tests are performed in the afternoon, some steroids, which have a large diurnal variation, may have an abnormal response when expressed as a multiple above baseline even though the stimulated serum levels never exceed the normal range.

Adrenal Steroid Response To ACTH In Normal Pediatric Subjects

Steroid Data Presented as Mean and Range	Premature Infants 26-28 Weeks (N=21)			Premature Infants 34-36 Weeks (N=12)			Infants 1-6 Months (N=15)		
	0 Min	60 Min	Delta	0 Min	60 Min	Delta	0 Min	60 Min	Delta
11-Desoxycortisol (ng/dL)	539 110-1376	831 206-2504	292 15-1128	165 70-455	243 81-645	78 40-190	53 10-200	177 101-392	124 5-366
17-OH-Pregnenolone (ng/dL)	1402	5176	3775	1242	3803	2561	320	1465	1144
	375-3559	2331- 11,440	1219-9799	559-2906	831-9760	346-8911	52-828	633-3286	229-3104
17-OH-Progesterone (ng/dL)	471	774	302	306	562	256	47	172	125
	124-841	285-1310	50-596	186-472	334-1725	18-1253	13-173	85-250	52-193
18-OH-Corticosterone (ng/dL)	218 10-670	453 35-1500	235 16-830	318 38-779	821 152-2183	530 114-2183	78 5-300	279 130-465	200 21-394
Aldosterone (ng/dL)	192 5-635	320 13-1046	128 8-517	178 12-736	316 42-1365	148 28-629	27 2-71	77 4.8-166	50 2.7-123
Androstenendione (ng/dL)	239 63-935	607 121-1323	329 <10-745	323 61-875	731 162-1453	369 <10-1144	– <10-48	24 <10-87	– <10-45
Corticosterone (ng/dL)	560 235-1108	4135 1667-8251	3574 1338-8016	1522 201-5030	7382 2240-11,900	5860 2039-10,141	581 78-2500	3720 2225-4974	3138 1149-4789
Cortisol (µg/dL)	6.0 1-11	19 6-52	13 4-41	14 3-34	35 16-76	21 6-44	12 3-22	38 27-50	26 19-41
DHEA (ng/dL)	*	*	*	*	*	*	*	*	*
DHEA Sulfate (µg/dL)	+	+	+	+	+	+	+	+	+
DOC (ng/dL)	47 20-105	105 44-320	58 17-215	44 28-78	59 28-95	17 1-67	20 7-48	75 40-158	55 13-144
Pregnenolone (ng/dL)	793 260-2104	1879 962-3179	1086 70-2673	585 203-1024	1218 637-1888	686 162-1685	60 10-150	220 100-359	160 20-282
Testosterone (F) (ng/dL)	11	**	**	**	**	**	4.4	**	**
	5-16						2-8		
Testosterone (M) (ng/dL)	91	**	**	**	**	**	156	**	**
	59-125						2-501		

*DHEA values unavailable for HPLC/MS-MS for ACTH response
+DHEA-S does not respond acutely to ACTH. For baseline values please refer to age-related reference ranges.
**Testosterone levels are not significantly changed by low dose ACTH stimulation. Baseline data are presented for reference purposes only.

Steroid Values In Normal Subjects

Premature Infants

ACTH response studies are summarized on 21 infants (12 males and 9 females) at 26-28 weeks gestation (4 days of life) and 12 infants (5 males and 7 females) at 34-36 weeks gestation (1 day of life). As previously reported,(1-4) the serum levels of most adrenal steroids in premature infants are strikingly elevated in relation to full-term and older infants. Some caution should be exercised in the interpretation of laboratory data in these patients to avoid confusion with congenital adrenal

hyperplasia. The D-5 steroids, DHEA, pregnenolone and 17-OH pregnenolone, are especially elevated, reflecting decreased 3B-HSD activity and increased fetal adrenal synthesis of estriol precursors. Serum levels of these steroids decrease progressively with age, so that values found in premature newborns at < 30 weeks, are considerably higher than those observed at 35 weeks and in full-term neonates. Serum levels then continue to decline in young infants and reach a plateau after one year. The finding of very high 17-OH pregnenolone levels in young infants has been evaluated further in our laboratory to determine whether the assay is measuring additional unknown steroids. Despite testing with a number of procedures commonly used to check method specificity, the values are unchanged, and we have not found any evidence that other steroids are contributing to these high results. The 17-OH pregnenolone values show a linear response when eluates of chromatograms are assayed at multiple doses. Results obtained with different 17-OH-pregenolone antisera are also basically equivalent. Similarly, values obtained with methods using column, high pressure liquid, or paper chromatography are not different. Oxidation of samples with permanganate followed by chromatography also does not lower results. Finally, peaks from paper chromatograms are homogeneous and show a constant specific activity after elution and RIA of multiple fractions.

Infants 1-12 Months

Stimulation studies were performed on 27 normal infants (16 males and 11 females) between the ages of 1 and 12 months. The results have been segregated into two groups, 1-6 months (N=15) and 6-12 months (N=12), in order to provide reference ranges for more restricted age groups and also because the levels of some steroids change significantly during this time period. The ACTH response values in this age group are significantly lower than those reported above for premature infants. The response of many R-4 adrenal steroids (progesterone, 17-OH-progesterone, cortisol, corticosterone, and 11-desoxycortisol) are similar to those observed in children and adults. The D-5 steroids and the mineralcorticoids, however, are much higher and more variable. The stimulated levels of aldosterone, 18-OHB and DOC decrease during the first year and continue to decline in older infants. The response levels of the D-5 steroids (17-OH-pregnenolone, DHEA and pregnenolone) which are strikingly elevated in premature infants and newborns, decline progressively during the first 12 months of life. The exaggerated response of 17-OH-pregnenolone (2,000-4,000 ng/dL) frequently seen in young infants decreases during the first few months, so that stimulated values above 500 ng/dL are seldom seen beyond one year. Similarly, DHEA values which frequently stimulate above 1000 ng/dL in very young infants decline during this period and do not stimulate above 150 ng/dL after one year of age. The finding of very high levels in premature infants followed by a long, slow decline in this age group suggests a progressive decrease in fetal adrenal activity.

Steroid Precursor To Product Ratios In Normal Pediatric Subjects

Steroid Precursor: Product Ratios Data Presented as Mean and Range	Premature Infants 26-28 Weeks (N=21)			Premature Infants 34-36 Weeks (N=12)			Infants 1-6 Months (N=15)		
	0 Min	60 Min	Delta	0 Min	60 Min	Delta	0 Min	60 Min	Delta
Pregnenolone	0.56	0.36	0.29	0.41	0.50	0.27	0.25	0.15	0.13
17-OH-Pregnenolone	0.25-0.70	0.25-5.0		0.17-0.73	0.17-1.3		0.1-0.7	0.03-0.25	
17-OH-Pregnenolone	2.9	6.7	3.8	3.9	6.7	7.0	6.1	8.5	9.1
17-OH-Progesterone	1.1-5.2	3.6-11		1.8-6.5	3.6-12		2-22	3-20	
17-OH-Progesterone	0.95	1.17	1.03	2.1	2.8	4.5	1.2	1.1	1.0
11-Desoxycortisol	0.35-2.4	0.25-2.1		0.9-4.8	0.8-4.2		0.4-3.1	0.5-2.0	
17-OH-Progesterone (ng/dL)	78	41	23	28	18	17	5.0	4.6	4.6
Cortisol (µg/dL)	18-220	15-170		9.0-71	6.2-51		0.8-12	0.2-6.9	
11-Desoxycortisol (ng/dL)	89	44	22	22	7.5	3.8	4.4	4.7	4.8
Cortisol (µg/dL)	25-300	10-189		2.9-115	3.1-26		0.8-10	2.4-10	
DOC X 100	8.3	2.5	1.6	2.8	0.7	0.29	10	2.0	1.7
Corticosterone X 100	2.3-25	1.2-7.0		1.1-5.5	0.4-0.9		0.5-30	0.7-4.9	
Corticosterone	2.6	9.1	15	4.8	8.9	11.0	8.9	16	15
18-OH-Corticosterone	1.6-43	3.9-58		2.9-11	4.4-28		3-26	7-30	
18-OH-Corticosterone	1.2	1.4	1.8	1.8	2.6	3.8	2.7	4.9	4.0
Aldosterone	1.0-4.5	0.8-2.6		1.1-10	1.2-11		1.3-5.0	2-13	
Corticosterone (ng/dL)	93	217	275	108	210	279	42	100	120
Cortisol (µg/dL)	35-198	34-800		42-242	140-375		8-120	37-146	

Adrenal Steroid Response To ACTH In Normal Pediatric Subjects

Steroid Data Presented as Mean and Range	Infants 6-12 Months (N=12)			Infants 1-2 Years (N=11)			Children 2-6 Years (N=15)		
	0 Min	60 Min	Delta	0 Min	60 Min	Delta	0 Min	60 Min	Delta
11-Desoxycortisol (ng/dL)	40 10-156	194 135-262	154 20-252	56 10-129	229 170-318	173 60-261	53 7-210	211 95-323	160 53-279
17-OH-Pregnenolone (ng/dL)	124 14-647	937 257-2173	813 221-1981	42 14-207	299 55-732	257 35-712	41 10-103	172 45-347	131 16-276
17-OH-Progesterone (ng/dL)	25 11-106	170 102-267	145 48-247	30 4-105	179 65-353	154 40-341	34 7-114	135 50-269	110 16-176
18-OH-Corticosterone (ng/dL)	62 5-310	244 120-471	192 57-371	78 20-155	210 120-325	133 80-208	30 7-74	182 110-350	153 33-332
Aldosterone (ng/dL)	16 2-39	54 5-94	38 3-55	18 5-43	44 28-85	21 7-53	11 2-22	29 13-50	18 6-41
Androstenendione (ng/dL)	– <10	– <10-47	– <10-28	– <10	– <10-37	– <10-31	– <10	– <10-35	– <10
Corticosterone (ng/dL)	559 78-3158	4003 2225-6529	3444 568-5115	695 135-2478	4002 2000-6315	3307 906-5134	454 159-2036	4418 1779-7554	3964 956-7301
Cortisol (µg/dL)	13 6-23	40 25-60	27 17-41	13 6-25	33 23-40	20 7-28	11 6-19	27 20-33	16 5-24
DHEA (ng/dL)	*	*	*	*	*	*	*	*	*
DHEA Sulfate (µg/dL)	+	+	+	+	+	+	+	+	+
DOC (ng/dL)	22 9-57	73 46-149	52 5-91	18 5-42	85 41-152	67 34-134	12 4-49	69 26-139	57 16-128
Pregnenolone (ng/dL)	43 12-137	159 75-294	116 43-282	33 10-93	89 44-135	55 4-113	35 17-50	69 34-99	35 15-75
Testosterone (F) (ng/dL)	3.1 2-8	**	**	**	**	**	**	**	**
Testosterone (M) (ng/dL)	4.5 2-8	**	**	4.0 2-8	**	**	4.0 2-8	**	**

*DHEA values unavailable for HPLC/MS-MS for ACTH response
+DHEA-S does not respond acutely to ACTH. For baseline values please refer to age-related reference ranges.
**Testosterone levels are not significantly changed by low dose ACTH stimulation. Baseline data are presented for reference purposes only.

Steroid Values In Normal Subjects

Children 1-6 Years

Response test results are summarized on 26 normal controls (14 boys, 12 girls) between 1 and 6 years old. Data are reported separately for 1-2 years (N=11) and 2-6 years (N=15). In general, there is little change in steroid levels throughout this age group. The only notable exception being 17-OH pregnenolone, which continues to fall during the first year and is still somewhat higher in children at 1 to 1.5 years than in older age groups.

Steroid Precursor To Product Ratios In Normal Pediatric Subjects

Steroid Precursor: Product Ratios Data Presented as Mean and Range	Infants 6-12 Months (N=12)			Infants 1-2 Years (N=11)			Children 2-6 Years (N=15)		
	0 Min	60 Min	Delta	0 Min	60 Min	Delta	0 Min	60 Min	Delta
Pregnenolone	0.53	0.2	0.14	0.9	0.5	0.20	1.1	0.5	0.3
17-OH-Pregnenolone	0.2-2.9	0.05-0.50		0.3-2.4	0.2-1.5		0.3-3.6	0.3-1.4	
17-OH-Pregnenolone	4.0	5.3	5.6	1.1	1.6	1.7	1.3	1.4	1.2
17-OH-Progesterone	1.5-10	1.6-12		0.4-2.5	0.5-3.3		0.3-3.0	0.45-2.6	
17-OH-Progesterone	0.8	0.9	1.1	0.7	0.8	0.9	1.1	0.7	0.7
11-Desoxycortisol	0.5-1.9	0.5-2.0		0.4-1.5	0.5-1.6		0.3-2.1	0.5-1.4	
17-OH-Progesterone (ng/dL)	1.7	4.0	5.3	1.9	5.5	7.7	3.1	5.2	6.9
Cortisol (µg/dL)	1.2-4.6	2.0-6.0		1.0-6.5	1.8-10		1.2-8.0	3.1-10	
11-Desoxycortisol (ng/dL)	2.3	4.6	5.7	4.0	7.2	8.6	4.6	7.9	10
Cortisol (µg/dL)	1.0-5.3	3.5-8.0		1.0-6.8	5-10		1.8-6.0	3.8-11	
DOC X 100	7.5	1.7	1.5	3.5	2.3	1.8	3.2	1.6	1.4
Corticosterone X100	3-18	1.2-2.6		2.0-7.0	1.2-4.5		1.2-5.6	0.9-3.3	
Corticosterone	8.7	18	18	10	19	25	19	24	25
18-OH-Corticosterone	4-20	10-33		2-32	12-27		5-36	14-50	
18-OH-Corticosterone	2.7	5.3	5.0	3.6	4.9	6.3	3.8	7.1	8.5
Aldosterone	1.4-3.9	3-12		1.3-5.0	3.3-7.0		1.2-6.0	1.9-15	
Corticosterone (ng/dL)	29	94	127	40	124	165	36	150	223
Cortisol (µg/dL)	10-137	45-146		15-157	99-150		17-46	71-259	

Adrenal Steroid Response To ACTH In Normal Pediatric Subjects

Steroid Data Presented as Mean and Range	Children 6-10 Years (N=18)			Early Puberty Males (N=14)			Late Puberty Males (N=15)		
	0 Min	60 Min	Delta	0 Min	60 Min	Delta	0 Min	60 Min	Delta
11-Desoxycortisol (ng/dL)	53 14-136	188 95-254	135 34-182	53 11-151	180 115-284	127 35-241	49 14-115	169 87-218	120 44-168
17-OH-Pregnenolone (ng/dL)	76 10-186	329 70-656	253 59-515	95 20-363	390 88-675	295 66-655	123 32-297	572 220-966	449 165-842
17-OH-Progesterone (ng/dL)	36 7-100	158 85-280	122 51-240	45 12-131	154 69-313	109 7-281	100 51-191	172 105-264	72 12-134
18-OH-Corticosterone (ng/dL)	35 16-72	183 95-289	140 79-240	25 5-73	152 68-196	127 63-186	30 14-62	120 73-206	93 21-198
Aldosterone (ng/dL)	9 4-21	25 14-42	16 5-35	7 2-14	23 10-33	16 7-22	7 3-14	22 13-32	15 7-25
Androstenendione (ng/dL)	– <10	19 <10-69	– <10-23	12 <10-52	30 <10-64	– <10	63 25-127	112 48-197	12 <10-95
Corticosterone (ng/dL)	483 155-1368	3911 2516-5648	3428 1958-5237	300 115-1219	3159 1472-4502	2859 998-4502	326 165-836	3022 1784-5060	2696 1074-4751
Cortisol (µg/dL)	11 5-16	25 20-31	15 10-20	11 4-17	23 15-36	14 7-32	10 5-15	23 18-28	13 5-21
DHEA (ng/dL)	*	*	*	*	*	*	*	*	*
DHEA Sulfate (µg/dL)	+	+	+	+	+	+	+	+	+
DOC (ng/dL)	10 4-17	48 22-120	38 16-70	9 2-15	38 12-74	29 7-44	8 5-13	30 19-46	23 7-36
Pregnenolone (ng/dL)	33 15-63	82 39-130	48 15-115	28 10-55	90 58-116	62 41-101	30 11-50	92 37-149	61 8-99
Testosterone (ng/dL)	5 2-19	**	**	161 20-310	**	**	501 278-702	**	**

*DHEA values unavailable for HPLC/MS-MS for ACTH response
+DHEA-S does not respond acutely to ACTH. For baseline values please refer to age-related reference ranges.
**Testosterone levels are not significantly changed by low dose ACTH stimulation. Baseline data are presented for reference purposes only.

Steroid Values In Normal Subjects

Children

Stimulation test data are reported for 18 normal controls (8 boys and 10 girls) between the ages of 6 and 11 years. The ACTH responsiveness of the D-5 steroids (17-OH-pregnenolone and DHEA) increase significantly during this period and continue to change throughout puberty. This normal physiological process results in a significant increase in D5/D4 steroid precursor/product ratios and should not be misinterpreted as a mild 3-beta HSD deficiency.

Pubertal Children

Studies are reported on 56 normal pubertal controls between the ages of 12 and 17 years. The subjects were separated by sex and tanner stage into the following groups:

Early Pubertal Females (Tanner Stage 2-3, N=12)
Late Pubertal Females (Tanner Stage 4-5, N=15)
Early Pubertal Males (Tanner Stage 2-3, N=14)
Late Pubertal Males (Tanner Stage 4-5, N=15)

Pubertal stages were assigned on the basis of breast development and pubic hair in females and genitalia and pubic hair in males. Results on female controls in the luteal phase of their cycle (as indicated by serum progesterone levels) were excluded from the data summary. The response of D-4 steroids in pubertal age groups is similar to prepubertal children and adults. The D-5 steroids which begin changing at adrenarche continue to increase throughout puberty. The ACTH response of DHEA and 17-OH pregnenolone in late puberty is considerably higher than the early pubertal ages. This increase is reflected in significant changes of the D5/D4 steroid ratios. The shift in D-5 steroid secretion is more pronounced in females. The stimulated levels of 17-OH pregnenolone and DHEA are significantly higher in late pubertal and adult females than in age equivalent male controls.

Steroid Precursor To Product Ratios In Normal Pediatric Subjects

Steroid Precursor: Product Ratios Data Presented as Mean and Range	Children 6-10 Years (N=18)			Early Puberty Males (N=14)			Late Puberty Males (N=15)		
	0 Min	60 Min	Delta	0 Min	60 Min	Delta	0 Min	60 Min	Delta
Pregnenolone	1.0	0.3	0.2	0.5	0.3	0.2	0.4	0.2	0.13
17-OH-Pregnenolone	0.2-2.8	0.16-0.85		0.1-1.7	0.15-1.0		0.1-1.1	0.1-0.3	
17-OH-Pregnenolone	1.8	2.4	2.0	1.9	2.8	2.7	1.2	3.4	6.8
17-OH-Progesterone	0.5-6.0	0.3-5.3		0.5-3.3	0.5-6.3		0.4-3.4	1.8-5.2	
17-OH-Progesterone	0.7	0.9	0.9	1.0	0.88	0.9	1.5	1.2	0.5
11-Desoxycortisol	0.2-2.1	0.5-1.6		0.5-2.0	0.4-2.1		0.8-3.7	0.6-2.7	
17-OH-Progesterone (ng/dL)	2.8	5.4	8.1	5.7	7.7	9.1	9.5	7.4	5.0
Cortisol (µg/dL)	0.8-5.0	2.8-9.0		2.0-13	3.6-16		5.0-19	4.0-11	
11-Desoxycortisol (ng/dL)	4.8	6.4	9.0	6.0	6.5	8.4	5.4	6.8	8.0
Cortisol (µg/dL)	1.2-9.0	2.8-9.0		2-11	4.0-11		1.8-12	3.4-11	
DOC X 100	2.4	1.2	1.0	2.6	1.3	1.0	2.5	1.2	1.0
Corticosterone X 100	1.0-4.0	0.6-2.1		1.3-5.1	0.7-2.0		1.3-4.9	0.6-2.2	
Corticosterone	15	21	24	12	21	18	11	22	28
18-OH-Corticosterone	8-27	16-28		3-28	11-33		7-21	16-30	
18-OH-Corticosterone	3.8	6.9	8.7	3.6	7.2	7.9	4.2	5.6	6.2
Aldosterone	2.6-7.1	5-12		2.0-5.7	3.4-13		3.0-5.6	3.9-9.6	
Corticosterone (ng/dL)	45	150	228	34	159	238	32	137	207
Cortisol (µg/dL)	15-91	100-225		18-95	64-232		13-90	70-213	

Adrenal Steroid Response To ACTH In Normal Pediatric Subjects

Steroid Data Presented as Mean and Range	Children 6-10 Years (N = 18)			Early Puberty Females (N = 12)			Late Puberty Females (N = 15)		
	0 Min	60 Min	Delta	0 Min	60 Min	Delta	0 Min	60 Min	Delta
11-Desoxycortisol (ng/dL)	53 14-136	188 95-254	135 34-182	52 15-130	171 90-251	120 34-233	53 15-115	154 78-237	101 38-182
17-OH-Pregnenolone (ng/dL)	76 10-186	329 70-656	253 59-515	132 33-451	527 251-756	395 108-683	208 44-542	1014 502-1402	806 438-1280
17-OH-Progesterone (ng/dL)	36 7-100	158 85-280	122 51-240	48 14-120	182 75-353	134 18-287	55 12-121	151 71-226	96 9-164
18-OH-Corticosterone (ng/dL)	35 16-72	183 95-289	140 79-240	39 10-82	150 69-196	110 58-173	23 9-68	131 82-242	108 61-204
Aldosterone (ng/dL)	9 4-21	25 14-42	16 5-35	10 2-20	22 12-31	12 3-21	7 3-15	17 10-34	10 2-26
Androstenendione (ng/dL)	– <10	19 <10-69	– <10-23	39 10-154	71 26-183	– <10-23	93 39-182	130 62-311	– <10-91
Corticosterone (ng/dL)	483 155-1368	3911 2516-5648	3428 1958-5237	246 152-598	2928 2257-4725	2704 2079-4504	241 162-389	2953 1723-5102	2712 1372-4713
Cortisol (µg/dL)	11 5-16	25 20-31	15 10-20	9 3-16	22 16-32	13 7-18	10 6-15	26 18-35	16 11-26
DHEA (ng/dL)	*	*	*	*	*	*	*	*	*
DHEA Sulfate (µg/dL)	+	+	+	+	+	+	+	+	+
DOC (ng/dL)	10 4-34	48 28-85	38 16-70	7 2-16	37 13-63	30 9-51	8 2-13	29 15-50	22 12-45
Pregnenolone (ng/dL)	33 15-63	82 39-170	48 15-115	39 15-84	89 33-139	50 5-95	41 19-87	146 76-218	105 38-191
Testosterone (ng/dL)	5 2-19	**	**	18 8-36	**	**	32 12-59	**	**

*DHEA values unavailable for HPLC/MS-MS for ACTH response
+DHEA-S does not respond acutely to ACTH. For baseline values please refer to age-related reference ranges.
**Testosterone levels are not significantly changed by low dose ACTH stimulation. Baseline data are presented for reference purposes only.

Steroid Values In Normal Subjects

Children

Stimulation test data are reported for 18 normal controls (8 boys and 10 girls) between the ages of 6 and 11 years. The ACTH responsiveness of the D-5 steroids (17-OH-pregnenolone and DHEA) increase significantly during this period and continue to change throughout puberty. This normal physiological process results in a significant increase in D5/D4 steroid precursor/product ratios and should not be misinterpreted as a mild 3-beta HSD deficiency.

Pubertal Children

Studies are reported on 56 normal pubertal controls between the ages of 12 and 17 years. The subjects were separated by sex and tanner stage into the following groups:

Early Pubertal Females (Tanner Stage 2-3, N=12)
Late Pubertal Females (Tanner Stage 4-5, N=15)
Early Pubertal Males (Tanner Stage 2-3, N=14)
Late Pubertal Males (Tanner Stage 4-5, N=15)

Pubertal stages were assigned on the basis of breast development and pubic hair in females and genitalia and pubic hair in males. Results on female controls in the luteal phase of their cycle (as indicated by serum progesterone levels) were excluded from the data summary. The response of D-4 steroids in pubertal age groups is similar to prepubertal children and adults. The D-5 steroids which begin changing at adrenarche continue to increase throughout puberty. The ACTH response of DHEA and 17-OH pregnenolone in late puberty is considerably higher than the early pubertal ages. This increase is reflected in significant changes of the D5/D4 steroid ratios. The shift in D-5 steroid secretion is more pronounced in females. The stimulated levels of 17-OH pregnenolone and DHEA are significantly higher in late pubertal and adult females than in age equivalent male controls.

Steroid Precursor To Product Ratios In Normal Pediatric Subjects

Steroid Precursor: Product Ratios Data Presented as Mean and Range	Children 6-10 Years (N=18)			Early Puberty Females (N=12)			Late Puberty Females (N=15)		
	0 Min	60 Min	Delta	0 Min	60 Min	Delta	0 Min	60 Min	Delta
Pregnenolone	1.0	0.3	0.2	0.43	0.2	0.12	0.3	0.15	0.13
17-OH-Pregnenolone	0.2-2.8	0.16-0.85		0.2-0.9	0.1-0.3		0.1-1.1	0.08-0.27	
17-OH-Pregnenolone	1.8	2.4	2.0	2.9	3.5	3.0	4.3	7.7	10
17-OH-Progesterone	0.5-6.0	0.3-5.3		0.7-8.0	1.5-7.0		0.8-10	3.0-17	
17-OH-Progesterone	0.7	0.9	0.9	0.9	1.1	1.1	1.8	1.0	1.0
11-Desoxycortisol	0.2-2.1	0.5-1.6		0.5-4.0	0.4-1.6		0.7-4.8	0.4-1.8	
17-OH-Progesterone (ng/dL)	2.8	5.4	8.1	5.2	7.7	10	6.1	5.9	5.6
Cortisol (µg/dL)	0.8-5.0	2.8-9.0		2.3-14	4.4-15		1.2-18	2.8-9.0	
11-Desoxycortisol (ng/dL)	4.8	6.4	9.0	5.8	8.1	9.0	5.5	5.5	5.6
Cortisol (µg/dL)	1.2-9.0	2.8-9.0		2.4-9.0	5.0-11.0		1.1-12	3.6-8.1	
DOC X 100	2.4	1.2	1.0	2.5	1.3	1.1	2.4	1.1	0.8
Corticosterone X 100	1.0-4.0	0.6-2.1		1.7-3.8	0.3-2.0		1.8-3.8	0.5-2.2	
Corticosterone	15	21	24	11	22	25	11	20	25
18-OH-Corticosterone	8-27	16-28		3-26	12-35		4-29	11-40	
18-OH-Corticosterone	3.8	6.9	8.7	3.9	7.2	9.1	3.7	7.8	10
Aldosterone	2.6-7.1	5-12		1.9-5.3	5.7-12		1.7-8.0	5-13	
Corticosterone (ng/dL)	45	150	228	33	142	208	28	114	169
Cortisol (µg/dL)	15-91	100-225		15-65	77-236		12-43	70-145	

Adrenal Steroid Response To ACTH In Normal Adult Subjects

Steroid Data Presented as Mean and Range	Adult Females Dex. Supp. (N=19)			Adult Females (N=19)			Adult Males (N=12)		
	0 Min	60 Min	Delta	0 Min	60 Min	Delta	0 Min	60 Min	Delta
11-Desoxycortisol (ng/dL)	15 10-40	125 60-205	110 40-186	65 15-158	140 65-237	75 30-205	40 20-65	132 73-214	94 17-175
17-OH-Pregnenolone (ng/dL)	20 10-24	768 240-1255	748 230-1255	138 48-320	871 290-1382	733 175-1300	100 20-187	558 240-1000	461 160-850
17-OH-Progesterone (ng/dL)	45 17-135	124 59-247	79 42-202	58 22-140	140 65-250	82 31-190	66 35-150	168 45-258	102 24-139
18-OH-Corticosterone (ng/dL)	20 6-43	153 68-243	133 41-218	23 9-58	138 52-225	125 34-206	27 13-48	133 85-249	106 49-201
Aldosterone (ng/dL)	6.6 1.2-21	20 7-33	13 3-22	8.4 2.4-25	20 6-28	12 2-19	7 3-11	17 7-26	10 0.3-18
Androstenendione (ng/dL)	81 37-148	207 79-289	88 <10-170	125 61-222	194 98-295	20 <10-137	86 50-210	159 78-285	32 <10-128
Corticosterone (ng/dL)	61 30-140	2743 1907-4268	2682 1704-4233	418 130-906	2890 1735-4752	2272 1280-4230	410 90-1204	3265 433-6590	2854 252-5386
Cortisol (µg/dL)	1.5 <1-2.4	23 16-36	21 16-33	11 7-21	25 17-39	14 8-24	11 7-15	24 19-31	13 8-18
DHEA (ng/dL)	*	*	*	*	*	*	*	*	*
DHEA Sulfate (µg/dL)	+	+	+	+	+	+	+	+	+
DOC (ng/dL)	4.6 2-15	33 9-87	28 6-78	7 3-19	29 12-90	22 5-70	6 3-13	26 14-38	20 2-32
Pregnenolone (ng/dL)	25 10-60	144 55-200	119 59-235	65 46-150	150 70-220	95 40-210	41 10-85	99 20-200	68 10-144
Testosterone (ng/dL)	24 10-47	**	**	35 15-54	**	**	543 430-675	**	**

*DHEA values unavailable for HPLC/MS-MS for ACTH response
+DHEA-S does not respond acutely to ACTH. For baseline values please refer to age-related reference ranges.
**Testosterone levels are not significantly changed by low dose ACTH stimulation. Baseline data are presented for reference purposes only.

Steroid Values In Normal Subjects

Adults

Stimulation studies are reported on 31 normal adult controls (19 females and 12 males) between the ages of 20 and 45 years. Data from an additional study is also reported in which the ACTH stimulation tests were done on 19 females after overnight suppression with 1 mg of dexamethasone. Samples were collected on women only during the follicular phase of the menstrual cycle.

Steroid Precursor To Product Ratios In Normal Adult Subjects

Steroid Precursor: Product Ratios Data Presented as Mean and Range	Adult Females Dex. Supp. (N=19)			Adult Females (N=19)			Adult Males (N=12)		
	0 Min	60 Min	Delta	0 Min	60 Min	Delta	0 Min	60 Min	Delta
Pregnenolone	2.0	0.20	0.16	0.5	0.2	0.14	0.48	0.14	0.15
17-OH-Pregnenolone	1.3-3.0	0.1-0.3		0.25-1.2	0.1-0.3		0.2-2.1	0.05-0.41	
17-OH-Pregnenolone	0.44	6.2	9.4	2.6	6.2	7.7	1.8	3.8	4.5
17-OH-Progesterone	0.2-0.8	3.7-11		1.7-6.0	3.7-11		0.5-5.8	1.6-7.0	
17-OH-Progesterone	2.5	1.0	0.8	0.9	1.0	1.3	1.9	1.2	1.1
11-Desoxycortisol	2.0-14	0.8-2.4		0.7-5.0	0.9-2.4		0.8-4.9	0.6-1.7	
17-OH-Progesterone (ng/dL)	23	5.4	3.8	4.2	5.4	6.8	6.1	6.6	8.0
Cortisol (µg/dL)	10-60	2.8-9.1		1.9-9.3	2.8-9.1		2-13	2-9	
11-Desoxycortisol (ng/dL)	9.5	5.4	5.1	5.0	5.4	5.3	3.5	5.5	7.2
Cortisol (µg/dL)	2.5-34	2.3-70		0.7-6.2	2.3-7.0		1.4-5.1	3.4-7.4	
DOC X 100	8	1.2	1.0	1.0	1.2	1.0	1.6	1.2	1.0
Corticosterone X 100	4-20	0.5-1.7		1.2-3.6	0.5-1.7		0.5-3.2	0.5-5.8	
Corticosterone	3.1	21	21	18	21	18	16	24	26
18-OH-Corticosterone	1.2-7.1	16-33		10-30	16-33		10-28	17-32	
18-OH-Corticosterone	3.0	7.7	10	2.1	7.7	10	2.9	6.3	11
Aldosterone	2.3-4.6	3.8-10		1.2-5.0	3.8-10		1.2-6.0	3-11	
Corticosterone (ng/dL)	32	119	127	39	119	162	38	131	219
Cortisol (µg/dL)	19-88	80-169		14-45	80-169		8-128	22-253	

Steroid Hormone Levels In Congenital Adrenal Hyperplasia

Steroid		21-OH Deficiency Classical (N=30)	21-OH Deficiency Late Onset (N=20)	11-OH Deficiency (N=20)	3-B-HSD Deficiency (N=6)	17-OH Deficiency (N=5)	CMO-II Deficiency (N=5)
Aldosterone (ng/dL)	**0 min**	2-90	2-20	1-18	4-200	0.7-4.0	1-12
	60 min	*	8-41	1-40	*	2.3-5.0	1-18
11-Desoxycortisol (ng/dL)	**0 min**	10-520	15-45	570-26,000	260-1800	15-65	10-180
	60 min	*	35-150	1200-50,000	*	25-94	145-360
Corticosterone (ng/dL)	**0 min**	45-1730	105-1110	70-1350	80-5700	9000-40,000	700-9000
	60 min	*	1470-4800	101-5800	*	20,000-57,000	3400-13,000
18-OH-Corticosterone (ng/dL)	**0 min**	2-145	25-70	2-45	16-380	100-300	85-2600
	60 min	*	65-310	6-70	*	300-550	130-4120
Cortisol (µg/dL)	**0 min**	1-18	3-12	1-26	1-15	0.5-3.0	3-16
	60 min	*	10-34	1-45	*	2.0-5.0	15-37
DHEA (ng/dL)	**0 min**	*	*	*	*	*	*
	60 min	*	*	*	*	*	*
DOC (ng/dL)	**0 min**	2-70	5-35	20-800	2-53	100-250	35-250
	60 min	*	20-75	225-2900	*	200-480	60-550
Pregnenolone (ng/dL)	**0 min**	190-3400	20-185	35-740	650-5900	300-800	*
	60 min	*	75-760	180-970	*	600-2400	*
17-OH-Pregnenolone (ng/dL)	**0 min**	600-22,000	20-510	125-1500	2200-48,000	20-50	*
	60 min	*	500-2890	430-3650	*	20-50	*
17-OH-Progesterone (ng/dL)	**0 min**	3000-120,000	90-2078	30-1250	335-4100	10-25	80-235
	60 min	*	1400-11,500	220-2360	*	25-50	140-490

Steroid Values In Patients With Adrenal Hyperplasia
Steroid data on a limited number of patients with different forms of CAH are summarized on subsequent pages. In most cases of CAH, the laboratory data are clearly abnormal and there is little confusion regarding interpretation. Precursor steroids in the pathway preceding the enzymatic block are usually very elevated. Those following the block may be low, normal, or high. The precursor product ratios at the location of the biosynthetic defect, however, are usually well beyond the values found in unaffected individuals. It should be noted that unstimulated levels of some steroids in patients with CAH exhibit wide fluctuations throughout the day. This phenomenon which probably reflects changes in endogenous ACTH secretion must be taken into account when evaluating patients with late onset or mild forms of adrenal hyperplasia. Because of these wide fluctuations, levels of key marker steroids may fall within the normal range when samples are drawn during the afternoon. Confusion with laboratory results or misdiagnosis in these cases can be avoided by using ACTH stimulation.

21-OH Deficiency
Serum 17-OH progesterone (17-OHP) is the standard laboratory marker for this disorder. In classical 21-OH deficiency, the values of 17-OHP are strikingly elevated (up to 2000 times normal) and there is little need for stimulation tests. In mild or late onset cases, ACTH stimulation is recommended to amplify defects. If ACTH is not used, samples should be drawn during the morning hours, since late afternoon 17-OHP values may fall into the normal range. Additionally, 21-deoxycortisol testing may be helpful in distinguishing late onset cases.

11-OH Deficiency
Serum 11-Desoxycortisol is highly elevated in patients with adrenal hyperplasia due to 11-OH deficiency and provides the most useful marker for this disorder. Stimulation tests are usually not required in young infants, but may be useful in evaluating older patients who have either a late onset or were not diagnosed until later in life. Nearly have of the cases summarized on page 113in this dataset were identified during evaluation for premature adrenarche.

17-OH Deficiency

Corticosterone and to a lesser extent DOC are highly elevated in these patients, and provide useful laboratory markers. The data on the 5 patients summarized above are relatively consistent. Cortisol, other 17-hydroxylated steroids and androgens are consistently very low. Steroids not hydroxylated at the 17-position are elevated, but the most pronounced increase appears to be in Corticosterone where values may be 100 times normal.

3-Beta-HSD Deficiency

Unlike most forms of CAH, the interpretation of laboratory data in late onset or mild 3-Beta-HSD deficiency is not clear, and there is considerable disagreement regarding the incidence and diagnostic criteria for this disorder. The ACH stimulated levels of 17-OH pregnenolone and DHEA which are key marker steroids for this disorder are widely scattered. There is no readily apparent separation of 3-HSD deficient patients from those who have elevated levels of adrenal steroids for other reasons. In children with premature adrenarche, the ACTH response of 17-OH pregnenolone and DHEA are elevated for age and are generally comparable with results seen in older pubertal children. It is not clear at the present time, however, whether this phenomenon results from a mild 3-Beta-HSD deficiency, or simply represents a premature expression of a normal physiological process. In adult women with hirsutism, the 17-OH pregnenolone and DHEA responses are often considerably higher than normal. There is a wide variation in values, however, and with the exception of a small number of cases, there is no clear separation of the data. For this reason, there is a tendency to assign the diagnosis on a statistical basis and it is not clear whether all these patients have 3-Beta-HSD deficiency.

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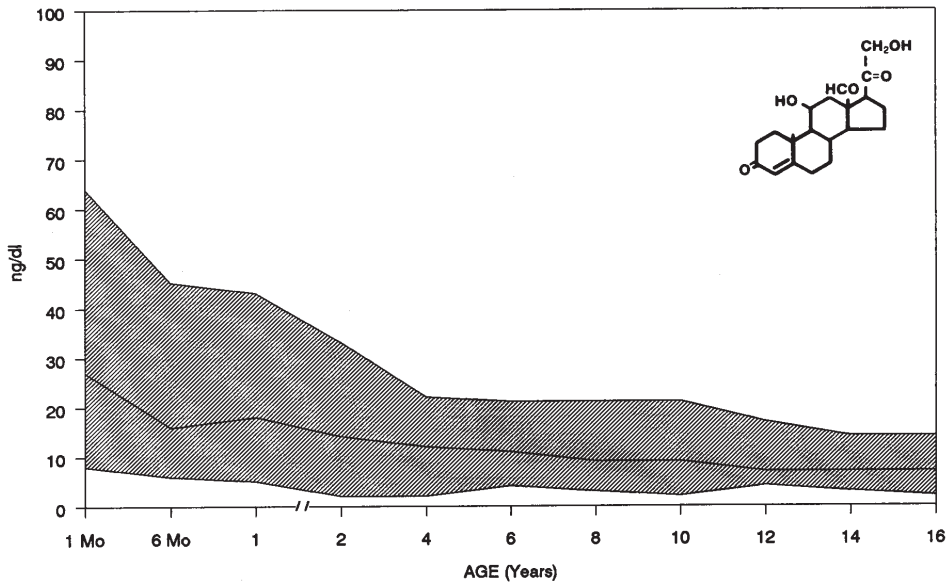
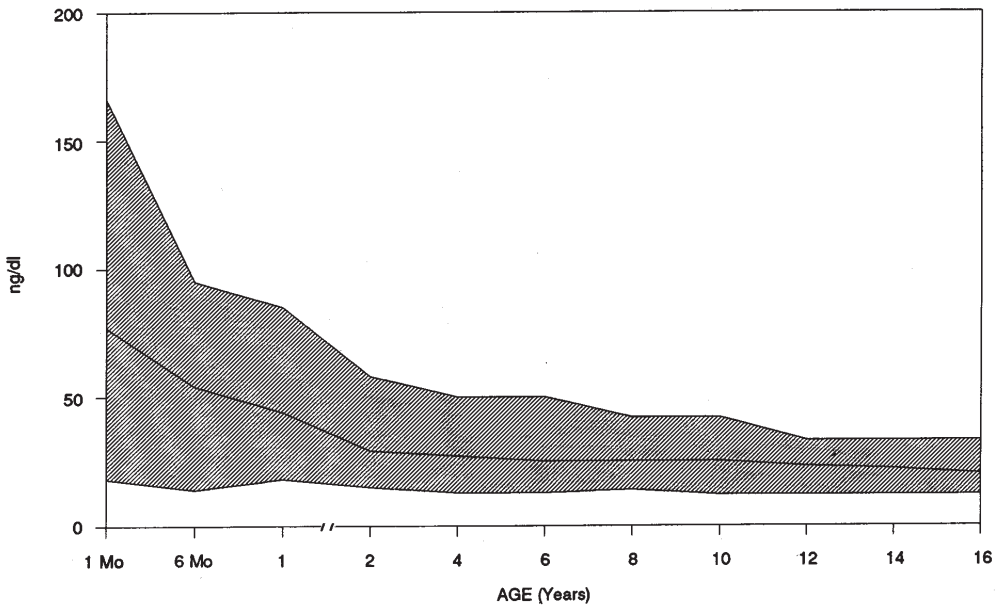
ALDOSTERONE
BASELINE/O MIN



ALDOSTERONE
ACTH STIMULATION/6O MIN



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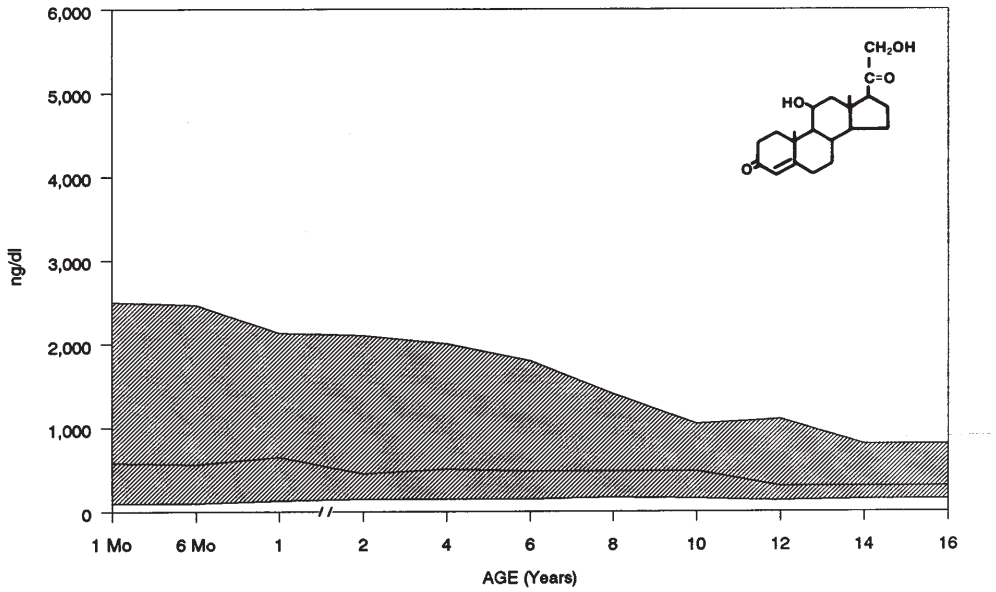

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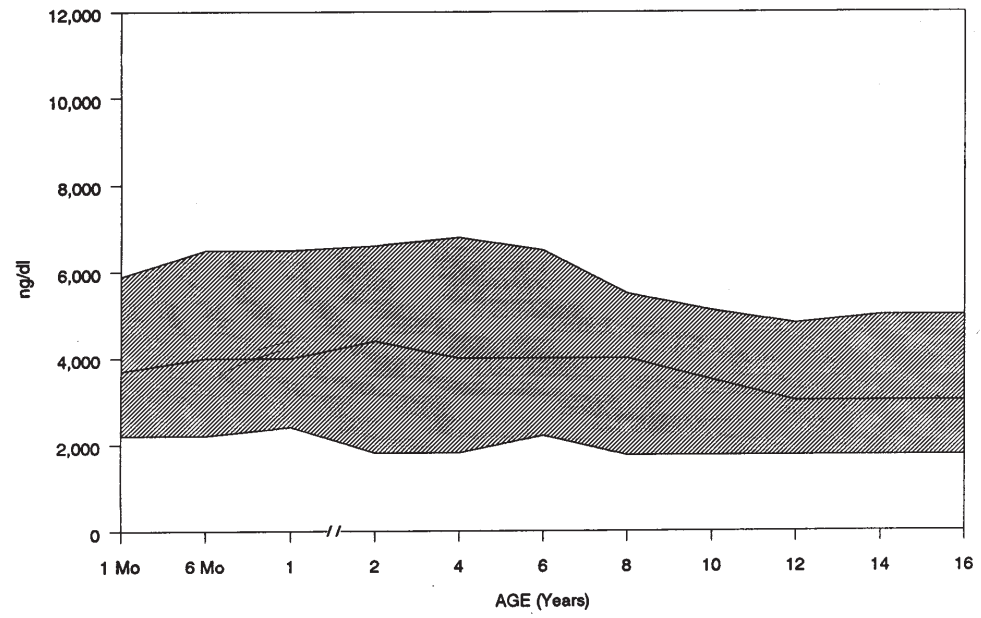

18-OH-CORTICOSTERONE
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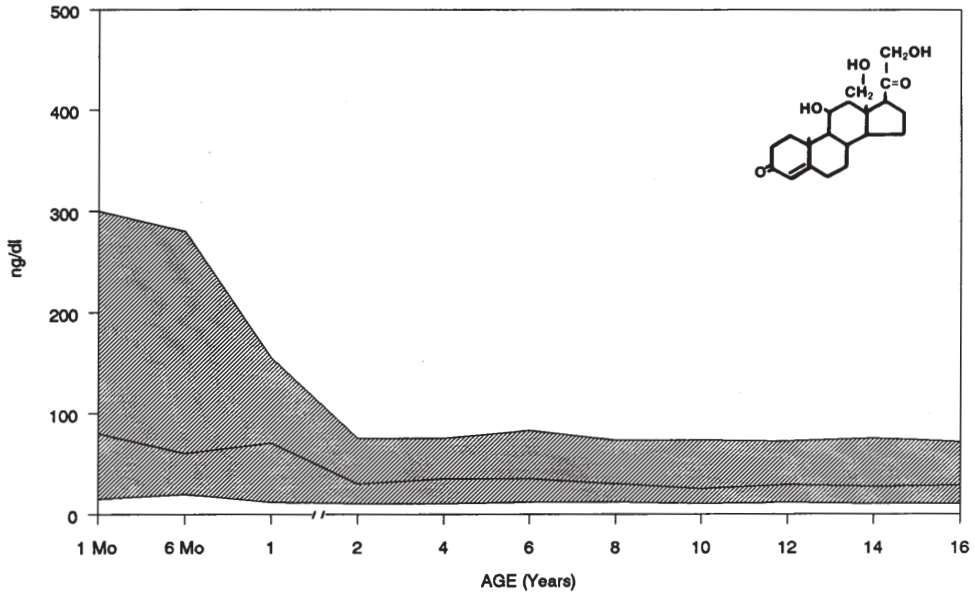

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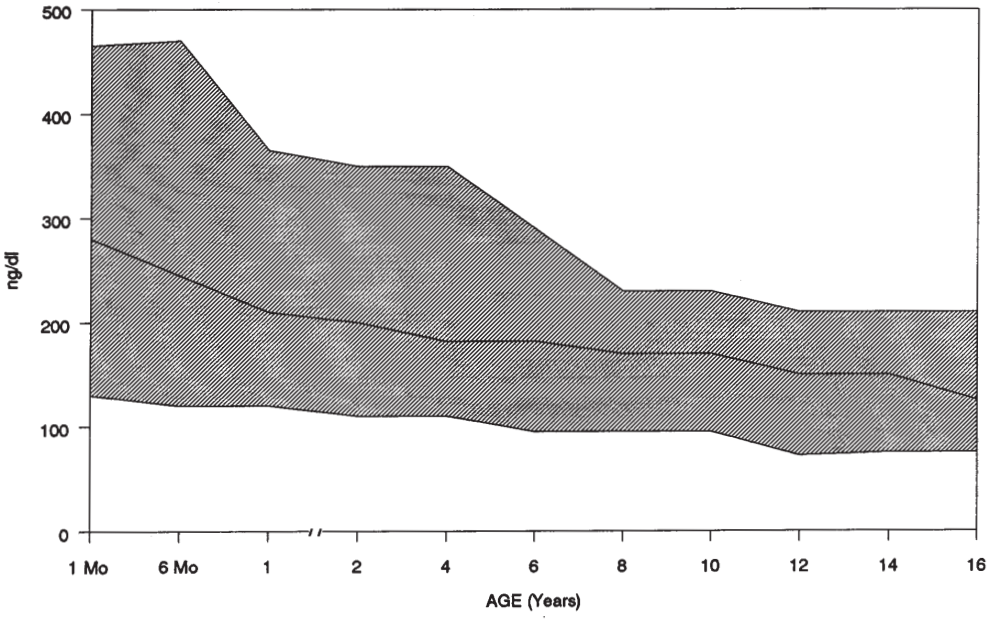

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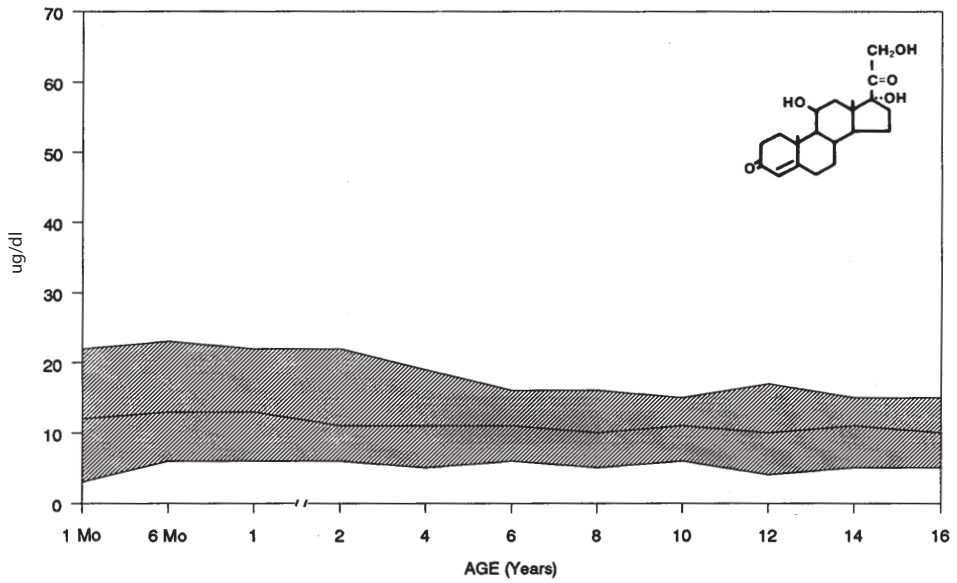

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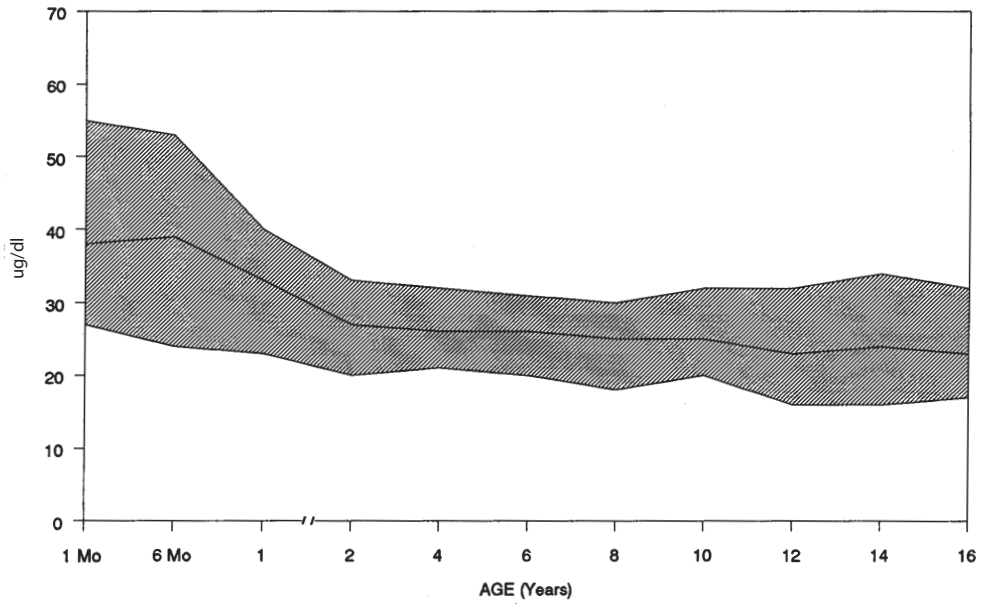

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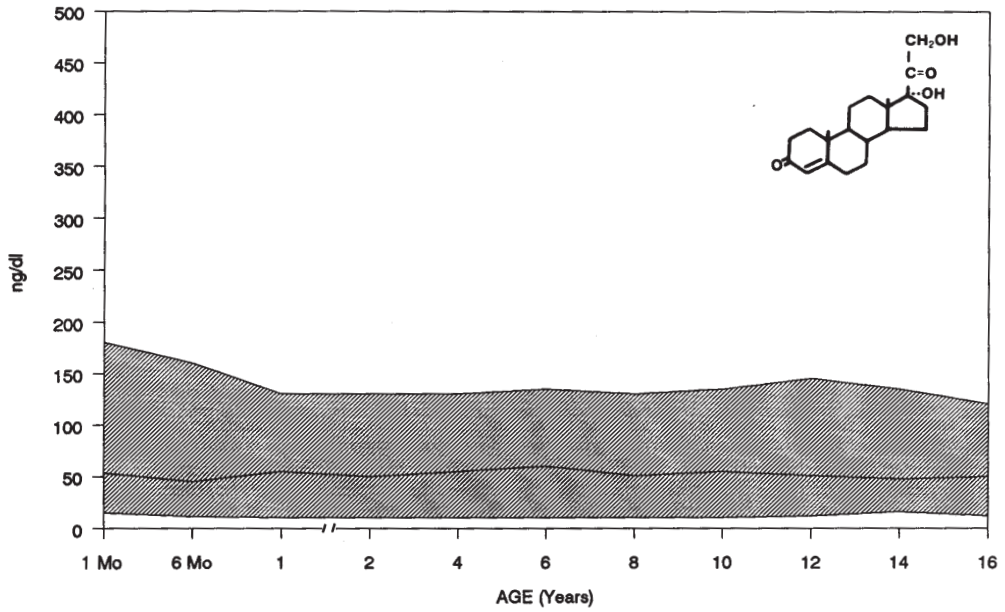

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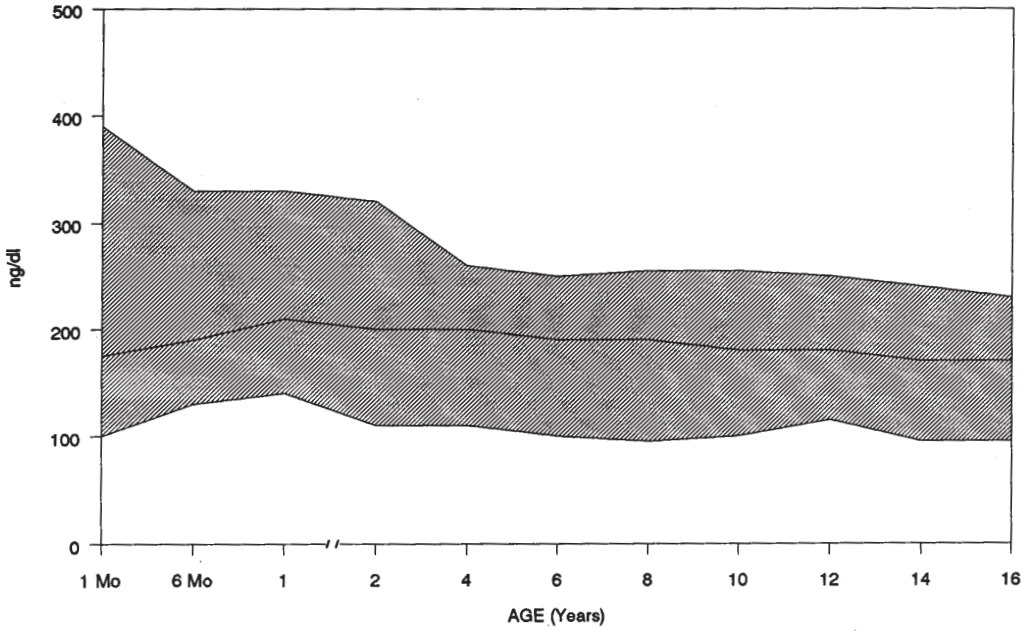

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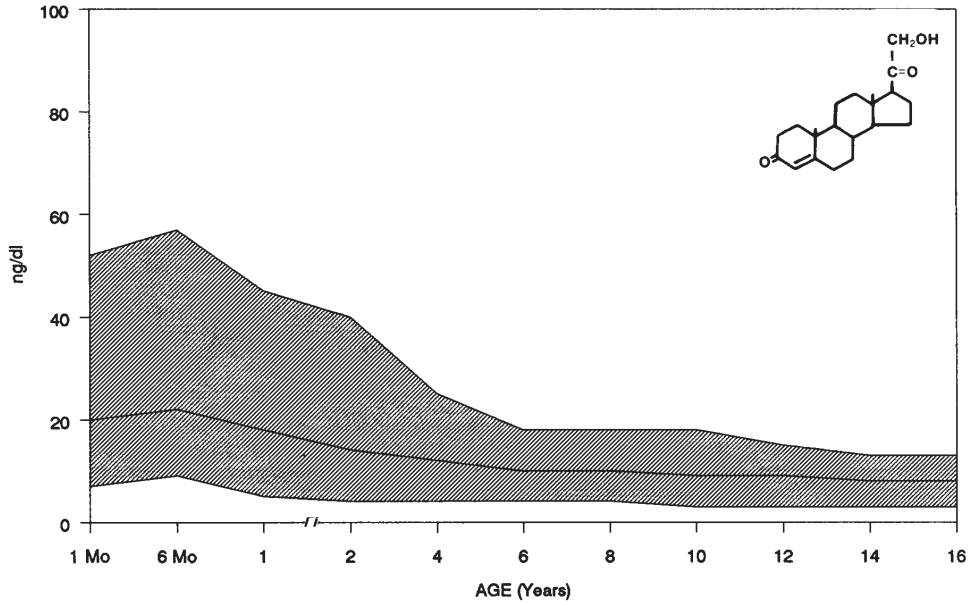

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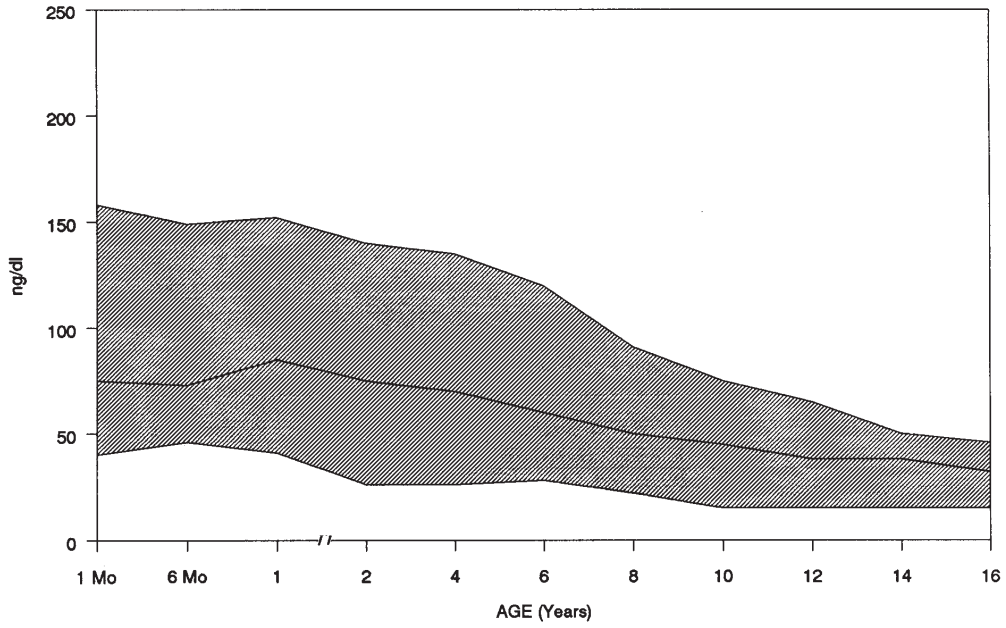

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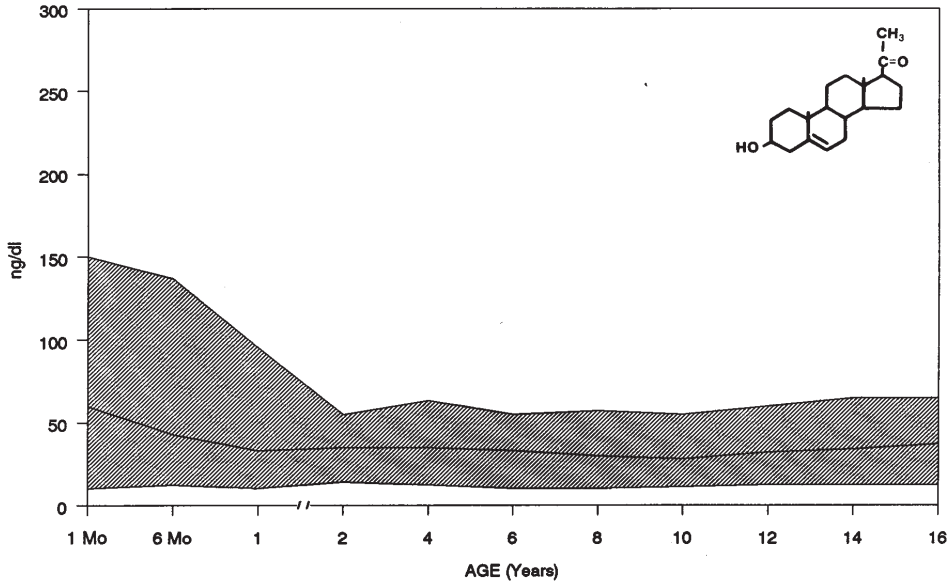

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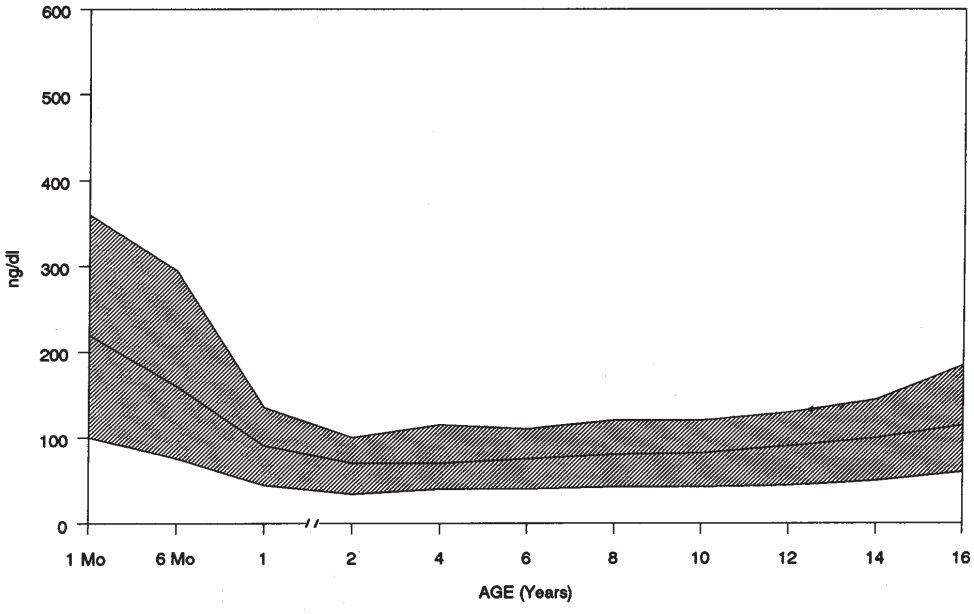

17-OH-PREGNENOLONE
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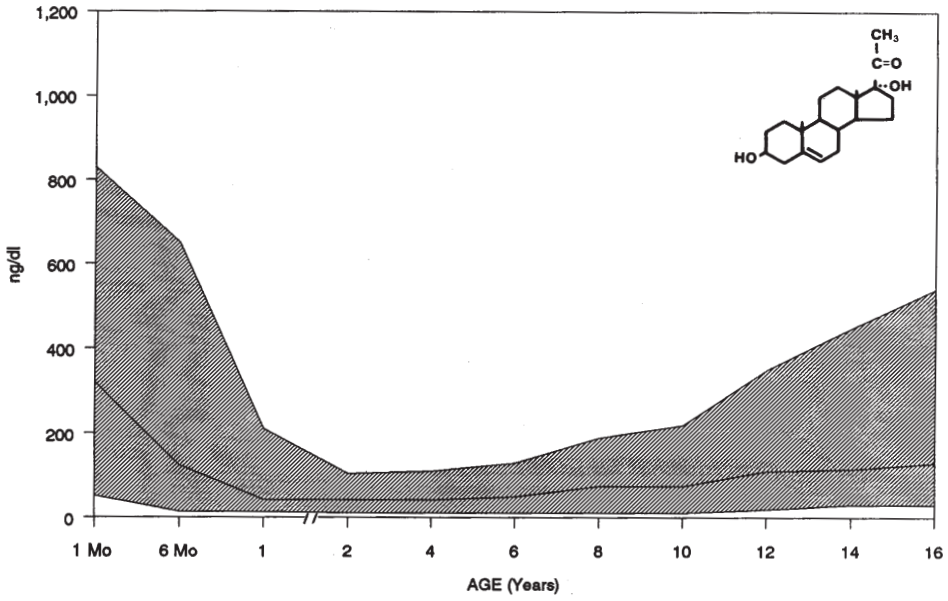

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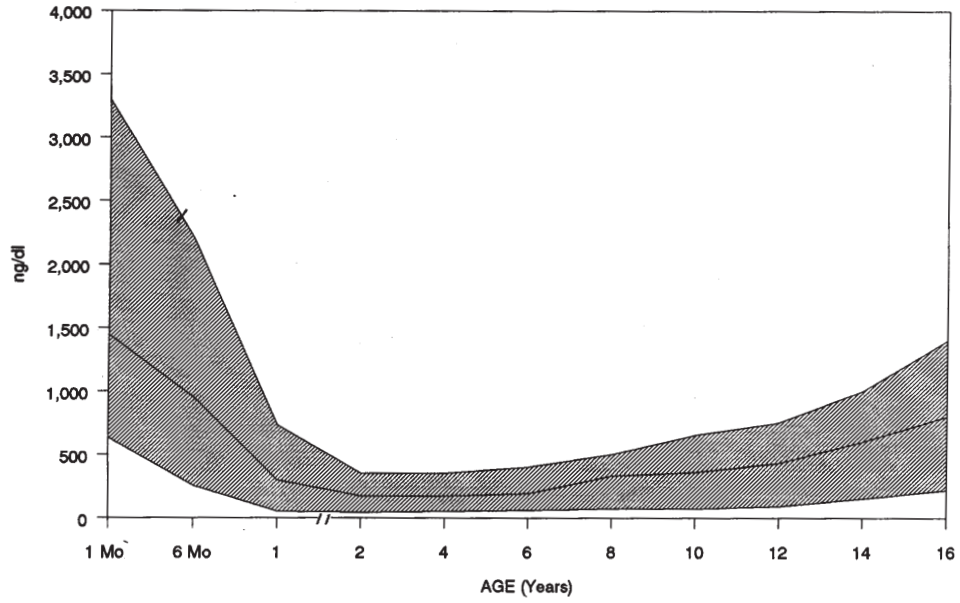

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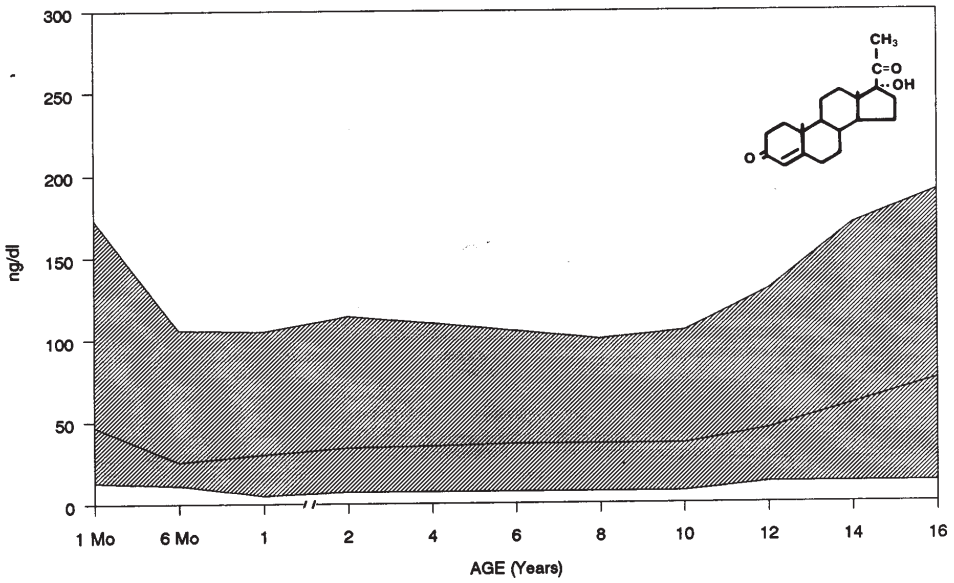

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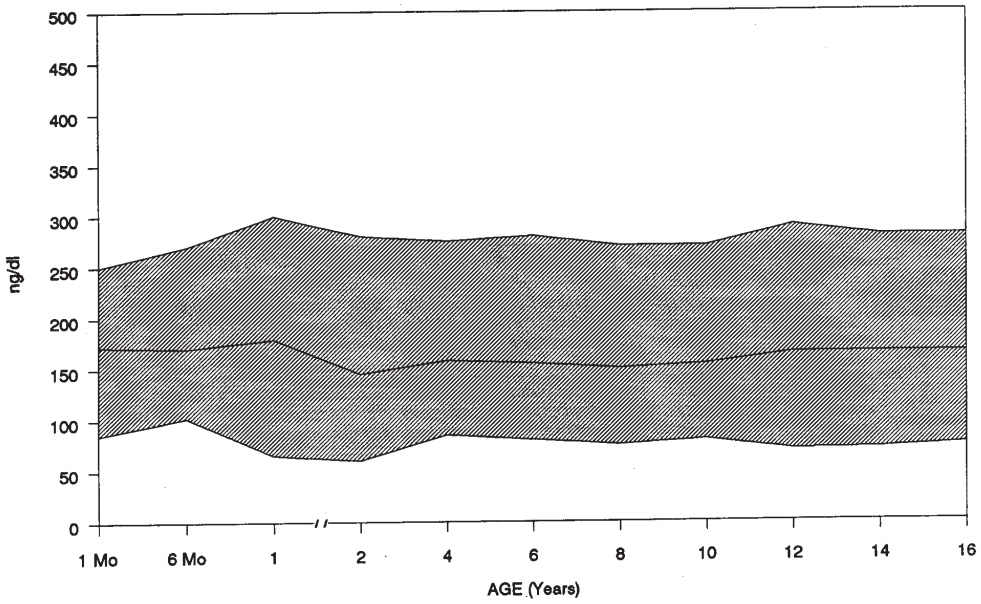

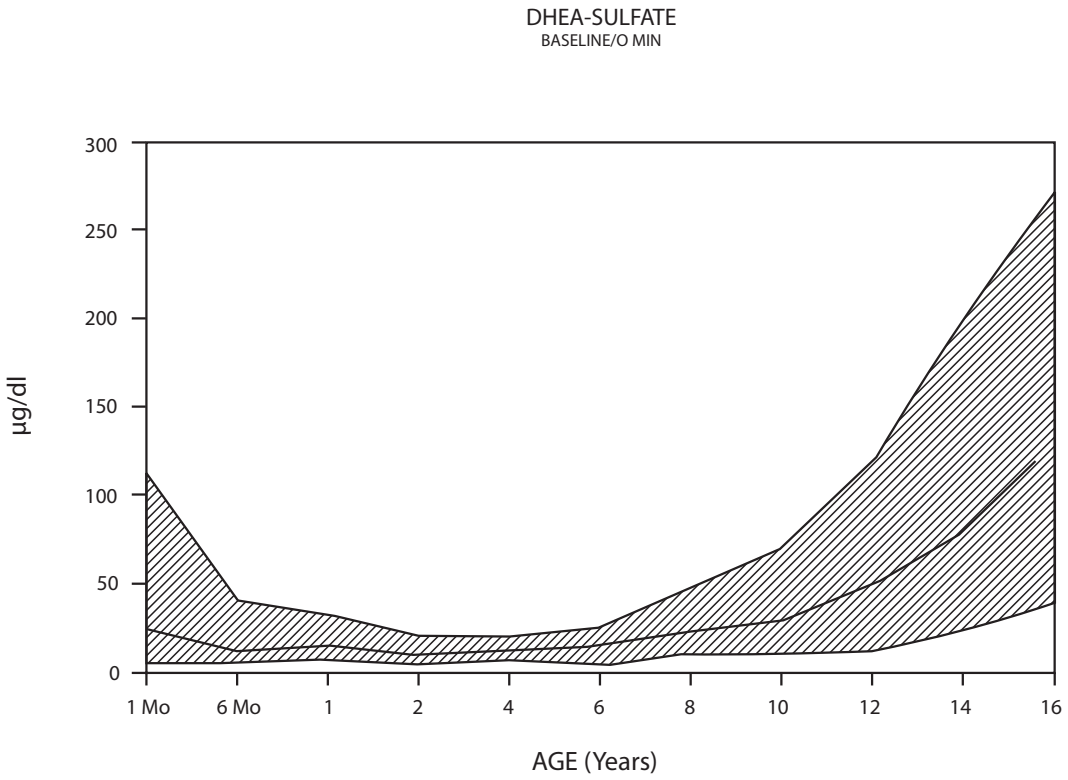

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Hormone	When You Know	Multiply By	To Find
ACTH (Corticotropin)	pg/mL	0.222	pmol/L
Antidiuretic Hormone (ADH)	pg/mL	0.923	pmol/L
Albumin	g/dL	10.0	g/L
Aldosterone, Serum	ng/dL	27.747	pmol/L
Aldosterone, Urine	µg/24 h	2.775	nmol/d
Aldosterone:Creatinine	µg/g	0.314	nmol:mmol
Androstanediol	ng/dL	344.3	nmol/L
Androstanediol Glucuronide	ng/dL	344.3	nmol/L
Androstenedione	ng/dL	0.035	nmol/L
Angiotensin I	pg/mL	0.772	pmol/L
Angiotensin II	pg/mL	0.957	pmol/L
Angiotensin I-converting Enzyme	U/L	0.017	ukat/L
Atrial Natriuretic Peptide (ANP)	pg/mL	0.325	pmol/L
C-Peptide	ng/mL	0.331	nmol/L
C-Peptide, Urine	ng/mL	0.331	nmol/L
Calcitonin	pg/mL	0.292	pmol/L
Calcium	mg/dL	0.25	mmol/L
Calcium, Urine	mg/24 h	0.025	mmol/d
Catecholamines, Urine	µg/24 h	5.911	nmol/d
Catecholamines:Creatinine	µg/g	0.669	nmol:mmol
Corticosterone	ng/dL	28.864	pmol/L
18-Hydroxy Cortisterone	ng/dL	27.594	pmol/L
Cortisol, Serum	µg/dL	27.588	nmol/L
Cortisol, Urine	µg/24 h	2.759	nmol/d
Cortisol:Creatinine	µg/dL	0.3121	nmol:mmol
Cortisone	µg/dL	0.278	nmol/L
Creatinine, Urine	mg/24 h	884	mmol/d
Cyclic AMP, Urine	nmol/mL	1.0	µmol/L
Cyclic AMP:Creatinine	nmol/mg	1.131	µmol:mol
Dehydroepiandrosterone (DHEA)	ng/dL	34.674	pmol/L

Hormone	When You Know	Multiply By	To Find
Dehydroepiandrosterone-Sulfate (DHEA-S)	µg/dL	27.211	nmol/L
18-Hydroxy-Deoxycorticosterone (18-OH DOC)	ng/dL	28.86	pmol/L
11-Desoxycortisol (Compound S)	ng/dL	28.868	pmol/L
21-Desoxycortisol	ng/dL	0.0289	nmol/L
Desoxycorticosterone (DOC)	ng/dL	30.2572	pmol/L
Dexamethasone	ng/dL	25.478	pmol/L
Dihydrotestosterone	ng/dL	34.435	pmol/L
Dopamine, Plasma	pg/mL	6.528	pmol/L
Dopamine, Urine	µg/24 h	6.53	nmol/d
Dopamine:Creatinine	µg/g	0.739	nmol:mmol
Endorphin, Beta	pg/mL	1.0	ng/L
Epinephrine, Plasma	pg/mL	5.459	pmol/L
Epinephrine, Urine	µg/24 h	5.459	nmol/d
Epinephrine:Creatinine	µg/g	0.618	µmol:mmol
Estradiol	pg/mL	3.671	pmol/L
Estriol	ng/mL	3.467	nmol/L
Estrogens, Serum	pg/mL	1.0	ng/L
Estrone	pg/mL	3.698	pmol/L
Estrone Sulfate	ng/dL	0.273	pmol/L
Folic Acid	ng/mL	2.266	nmol/L
Follicle-stimulating Hormone (FSH)	mIU/mL	1.0	IU/L
Follicle-stimulating Hormone, Urine	IU/24 h	1.0	IU/d
FSH:Creatinine	IU/g	0.113	IU:mol
Gastrin	pg/mL	0.481	pmol/L
Glucagon	pg/mL	1.0	ng/L
Growth Hormone	ng/mL	1.0	µg/L
Human Chorionic Gonadotrophin (hCG)	mIU/mL	1.0	IU/L
hCG, Urine	mIU/mL	1.0	IU/L

S.I. Unit Conversion Table

Hormone	When You Know	Multiply By	To Find
5-Hydroxy-Indoleacetic Acid (5-HIAA), Urine	mg/24 h	5.236	µmol/d
5-HIAA:Creatinine	mg/g	0.592	µmol:mmol
Homovanillic Acid (HVA), Urine	mg/24 h	5.489	µmol/d
HVA:Creatinine	µg/g	0.621	µmol:mmol
IGF I (Somatomedin C)	ng/mL	0.131	nmol/L
IGF II	ng/mL	0.133	nmol/L
Inhibin	U/mL	1.0	arb units/L
Insulin	µIU/mL	6.945	pmol/L
Luteinizing Hormone (LH)	mlU/mL	1.0	lU/L
Metanephrine, Urine	µg/24 h	0.005	µmol/d
Metanephrines, Total Urine	µg/24 h	5.258	nmol/d
Metanephrines, Total:Creatinine	µg/mg	0.574	mmol:mol
Methoxytyramine, Urine	µg/24 h	5.981	nmol/d
Norepinephrine, Plasma	pg/mL	5.914	pmol/L
Norepinephrine, Urine	µg/24 h	5.914	nmol/d
Norepinephrine:Creatinine	µg/g	0.669	nmol:mmol
Normetanephrine, Urine	µg/24 h	0.005	µmol/d
Normetanephrine:Creatinine	µg/g	0.618	µmol:mol
Osteocalcin	ng/mL	0.171	nmol/L
Parathyroid Hormone (PTH)	pg/mL	0.106	pmol/L
Prednisolone	ng/dL	27.739	pmol/L
Prednisone	ng/dL	27.902	pmol/L
Pregnenolone	ng/dL	0.032	nmol/L
17-Hydroxy-Pregnenolone	ng/dL	0.03	nmol/L
Progesterone	ng/mL	3.18	nmol/L
17-Hydroxy-Progesterone	ng/dL	0.03	nmol/L
Prolactin	ng/mL	43.478	pmol/L
Renin (Plasma Renin Activity)	ng/mL/h	0.278	ng/L/s
Reverse T_3	ng/dL	0.015	nmol/L
Secretin	pg/mL	0.327	pmol/L

Hormone	When You Know	Multiply By	To Find
Somatostatin-14	pg/mL	0.611	pmol/L
Somatostatin-28	pg/mL	0.305	pmol/L
Sex Hormone-binding Globulin (SHBG) (Binding Capacity)	nmol/L	1.0	nmol/L
Testosterone	ng/dL	0.035	nmol/L
Testosterone, Free	pg/mL	3.467	pmol/L
Testosterone, Urine	µg/24 h	3.467	nmol/d
Testosterone:Creatinine	µg/g	0.392	nmol:mmol
Thyroglobulin	ng/mL	1.0	µg/L
Thyroid-stimulating Hormone (TSH)	µIU/mL	1.0	µIU/mL
Thyroxine (T_4)	µg/dL	12.871	nmol/L
Thyroxine-binding Globulin	µg/mL	17.094	nmol/L
Thyrotropin-releasing Hormone (TRH)	pg/mL	2.762	pmol/L
Triiodothyronine (T_3)	ng/dL	0.015	nmol/L
Vanillylmandelic Acid (VMA), Urine	mg/24 h	5.05	µmol/d
VMA:Creatinine	mg/g	0.571	mmol:mmol
Vitamin B-12	pg/mL	0.738	pmol/L
25-Hydroxy-Vitamin D	ng/mL	2.521	nmol/L
1,25-Dihydroxy-Vitamin D	pg/mL	2.402	pmol/L

Test Name	Test No.	CPT Code(s)	Methodology	Specimen	Container	Storage	Result TAT
11-Hydroxytestosterone	504680	82542	HPLC/MS-MS	2 mL Serum (0.6 mL Minimum)	Red-top, Green-top (heparin), or lavender-top (EDTA) tube	**Frozen**	3-12 days
11-Hydroxyandrostenedione	504677	82542	HPLC/MS-MS	2 mL Serum (0.6 mL Minimum)	Red-top, Green-top (heparin), or lavender-top (EDTA) tube	**Frozen**	3-12 days
11-Ketotestosterone	504674	82542	HPLC/MS-MS	2 mL Serum (0.6 mL Minimum)	Red-top, Green-top (heparin), or lavender-top (EDTA) tube	**Frozen**	3-12 days
11-Oxoandrogens Panel	504683	82542	HPLC/MS-MS	2 mL Serum (0.6 mL Minimum)	Red-top, Green-top (heparin), or lavender-top (EDTA) tube	**Frozen**	3-12 days
21-Deoxycortisol	504045	82542	HPLC/MS-MS	1.0 mL Serum (preferred) or plasma Minimum: 0.3 mL	Red-top, gel-barrier, or lavender-top (EDTA) tube	**Frozen**	6-10 days
21-Hydroxylase Autoantibodies	504805	83516	ELISA	0.5 mL Serum Minimum: 0.2 mL	Red-top, or gel-barrier tube	Ambient Refrigerated or **frozen**	3-10 days
24-Hydroxylase Deficiency Screen, LC/MS-MS	504225	82652	HPLC/MS-MS	1 mL Serum (preferred) or plasma Minimum: 0.3 mL	Red-top, gel-barrier, lavender-top (EDTA), or green-top (heparin) tube	Ambient or refrigerated	
3-Epi-Vitamin D, 25-Hydroxy	504240	82306	HPLC/MS-MS	0.6 mL Serum (preferred) or EDTA plasma Minimum: 0.3 mL	Red-top, or Lavender-top (EDTA) tube	Refrigerated or **frozen**	9-15 days
Adrenal Androgen Profile (Androstenedione, DHEA Sulfate, Testosterone)	503198	82157; 82627; 84403	HPLC/MS-MS	2 mL Serum Minimum: 1 mL	Red-top tube only	Refrigerated (same day) or **frozen**	2-4 days
Adrenocorticotropic Hormone (ACTH)	500471	82024	ICMA	1 mL EDTA plasma Minimum: 0.3 mL	Lavender-top (EDTA) tube, chilled	**Frozen**	3-5 days
Aldosterone, Mass Spectrometry	500467	82088	HPLC/MS-MS	1 mL Serum or plasma Minimum: 0.3 mL	Red-top, Gel-barrier, Lavender-top (EDTA), or green-top (heparin) tube	Refrigerated or **frozen**	5-10 days
Alpha Reductase Profile, 5- (Dihydrotestosterone, Testosterone)	502682	82642; 84403	See individual assays	1.5 mL Serum Minimum: 1 mL	Gel-barrier tube, lavender-top (EDTA) tube, or green-top (heparin) tube	Refrigerated or **frozen**	2-7 days
Alpha Subunit, Free	140269	83520	ICMA	1 mL Serum Minimum: 0.25 mL	Red-top tube, gel-barrier tube, or lavender-top (EDTA) tube	**Frozen**	6-10 days

Test Name	Test No.	CPT Code(s)	Methodology	Specimen	Container	Storage	Result TAT
Amenorrhea Test Group (Estradiol, Follicle-stimulating Hormone, Luteinizing Hormone, Prolactin)	501064	82670; 83001; 83002; 84146	See individual assays	4 mL Serum Minimum: 2 mL	Red-top, or gel-barrier tube	Refrigerated (same day) or **frozen**	7-15 days
Androstanediol Glucuronide, LCMS	500881	82154	HPLC/MS-MS	1 mL Serum or EDTA plasma Minimum: 0.5 mL	Red-top, gel-barrier, or lavender-top (EDTA) tube	Ambient or refrigerated (preferred)	8-15 days
Androstenedione, Mass Spectrometry	500152	82157	HPLC/MS-MS	0.5 mL Serum or EDTA plasma Minimum: 0.2 mL	Red-top tube only, or lavender-top (EDTA) tube, or green-top (heparin) tube; Do not use gel-barrier tube	Refrigerated or **frozen**	5-10 days
Androsterone, Serum, LCMS	504005	82160	HPLC/MS-MS	1 mL Serum or EDTA plasma Minimum: 0.4 mL	Red-top tube only, or lavender-top (EDTA) tube; Do not use gel-barrier tube	**Frozen**	8-12 days
Anti-Müllerian Hormone (AMH), Serum	500183	82397	ECL	1 mL Serum Minimum: 0.2 mL	Red-top, or gel-barrier tube	Refrigerated or **frozen**	4-10 days
Antithyroglobulin Antibodies (Anti-Tg)	500555	86800	ICMA	0.5 mL Serum Minimum: 0.2 mL	Red-top, or gel-barrier tube	Ambient (same day) or **frozen**	3-5 days
Beta-Hydroxybutyrate	503610	82010	Spectrophotometry	1 mL Serum (preferred) or plasma Minimum: 0.3 mL	Red-top, gel-barrier, lavender-top (EDTA) tube, or green-top (heparin) tube	**Frozen**	3-10 days
CAH 21-Hydroxylase (CYP21) Mutation	500768	81402	Polymerase chain reaction (PCR), multiplex mini-sequencing, fragment analysis	3 mL Whole blood Minimum: 1 mL	Lavender-top (EDTA) tube	Ambient; do not freeze	17-28 days
Calcitonin	500636	82308	ICMA	1 mL Serum Minimum: 0.5 mL	Gel-barrier tube, red-top tube, or green-top (heparin) tube	**Frozen**	2-9 days
Calcium	501878	82310	Colorimetric	1 mL Serum or heparin plasma Minimum: 0.5 mL	Red-top, gel-barrier, or green-top (heparin) tube	Ambient or refrigerated	1-3 days
Calcium-Sensing Receptor (CASR) Gene Sequencing Analysis	504513	81405	Polymerase chain reaction (PCR) and Sanger sequencing of targeted CASR exons, gel electrophoresis	3 mL Whole blood Minimum: 1 mL	Lavender-top (EDTA) tube, or yellow-top (ACD) tube	Ambient	14-28 days
Catecholamine Test Group, Urine (Total Catecholamines, Total Metanephrines, VMA, Creatinine)	500837	82384; 82570; 83835; 84585	See individual assays	50 mL aliquot Urine (24-hour, well-mixed) Minimum: 10 mL aliquot	Urine container, 24-hour, with 25 mL 6N HCl preservative	Refrigerated or **frozen** (preferred)	2-7 days

Test Name	Test No.	CPT Code(s)	Methodology	Specimen	Container	Storage	Result TAT
Catecholamines, Fractionated, Urine (Includes Creatinine) (Epinephrine, Norepinephrine, Creatinine)	500520	82384; 82570	HPLC/MS-MS; colorometric	50 mL aliquot Urine (24-hour, well-mixed) Minimum: 5 mL aliquot	Urine container, 24-hour, with 25 mL 6N HCl preservative	Refrigerated or **frozen** (preferred)	2-7 days
Catecholamines, Total, Urine (Includes Creatinine)	500473	82384; 82570	HPLC/MS-MS; colorometric	50 mL aliquot Urine (24-hour, well-mixed) Minimum: 5 mL aliquot	Urine container, 24-hour, with 25 mL 6N HCl preservative	Refrigerated or **frozen** (preferred)	2-7 days
Chorionic Gonadotropin, Human (Beta-hCG), Quantitative	501676	84702	ICMA	0.5 mL Serum Minimum: 0.2 mL	Red-top, or gel-barrier tube	Refrigerated or **frozen**	3-5 days
Comprehensive GlycoMark® & A1C Profile (Hemoglobin A1c, GlycoMark®)	502226	83036; 84378	Roche Tina Quant, enzymatic	3 mL whole blood and 1 mL serum or EDTA plasma Minimum: 1 mL whole blood and 0.75 mL serum or EDTA plasma	Lavender-top (EDTA) whole blood and Red-top, or gel-barrier serum, or lavender-top (EDTA) plasma	Ambient or refrigerated	3-10 days
Congenital Adrenal Hyperplasia (CAH) Pediatric Profile 1, 21-OH Deficiency Screen (Androstenedione, Cortisol, DHEA, 17-OH-Progesterone, Testosterone)	501568	82157; 82533; 82626; 83498; 84403	HPLC/MS-MS	2 mL Serum Minimum: 1 mL	Red-top tube only; Do not use gel-barrier tube	Refrigerated (same day) or **frozen**	6-9 days
Congenital Adrenal Hyperplasia (CAH) Pediatric Profile 2, 11-OH Deficiency Screen (Androstenedione, Specific S, Cortisol, DHEA, 17-OH-Progesterone, Testosterone)	500176	82157; 82533; 82626; 82634; 83498; 84403	HPLC/MS-MS	2.5 mL Serum Minimum: 1.2 mL	Red-top tube only; Do not use gel-barrier tube	Refrigerated (same day) or **frozen**	6-9 days
Congenital Adrenal Hyperplasia (CAH) Pediatric Profile 3, 17-OH-Deficiency Screen (Corticosterone, Cortisol, DHEA, 17-OH-Progesterone, Progesterone)	502350	82528; 82533; 82626; 84144; 83498	HPLC/MS-MS	2 mL Serum Minimum: 0.7 mL	Red-top tube only; Do not use gel-barrier tube	Refrigerated (same day) or **frozen**	6-9 days
Congenital Adrenal Hyperplasia (CAH) Pediatric Profile 4, 3B-HSD Deficiency Screen (Androstenedione, Cortisol, DHEA, 17-OH-Pregnenolone, 17-OH-Progesterone)	501023	82157; 82533; 82626; 83498; 84143	HPLC/MS-MS	1.5 mL Serum Minimum: 0.8 mL	Red-top tube only; Do not use gel-barrier tube	Refrigerated (same day) or **frozen**	6-9 days
Congenital Adrenal Hyperplasia (CAH) Pediatric Profile 5, 17, 20 Desmolase Deficiency (Androstenedione, Cortisol, DHEA, 17-OH-Pregnenolone, Progesterone, 17-OH-Progesterone, Testosterone)	502348	82157; 82533; 82626; 83498; 84143; 84144; 84403	HPLC/MS-MS	3 mL Serum Minimum: 0.2 mL	Red-top tube only; Do not use gel-barrier tube	Refrigerated (same day) or **frozen**	6-9 days

Test Name	Test No.	CPT Code(s)	Methodology	Specimen	Container	Storage	Result TAT
Congenital Adrenal Hyperplasia (CAH) Pediatric Profile 6: Comprehensive Screen (Androstenedione, Specific S, Cortisol, DHEA, DOC, 17-OH-Pregnenolone, Progesterone, 17-OH-Progesterone, Testosterone)	500175	82157; 82533; 82626; 82633; 82634; 83498; 84143; 84144; 84403	HPLC/MS-MS	3.5 mL Serum Minimum: 2 mL	Red-top tube only; Do not use gel-barrier tube	Refrigerated (same day) or **frozen**	8-15 days
Congenital Adrenal Hyperplasia (CAH) Pediatric Profile 7, Treatment Profile (Androstenedione, 17-OH-Progesterone, Testosterone)	500166	82157; 83498; 84403	HPLC/MS-MS	1.5 mL Serum Minimum: 0.7 mL	Red-top tube only; Do not use gel-barrier tube	Ambient (same day) or **frozen**	5-15 days
Congenital Adrenal Hyperplasia (CAH) Test Group I Adult (Late-onset) (Androstenedione, Cortisol, Specific S, DHEA, 17-OH-Progesterone)	503192	82157; 82533; 82626; 82634; 83498	HPLC/MS-MS	2.3 mL serum Minimum 1.4 mL	Red-top tube only; Do not use gel-barrier tube	Refrigerated (same day) or **frozen**	6-9 days
Congenital Adrenal Hyperplasia (CAH) Test Group II Adult (Late-onset) (Androstenedione, Cortisol, DHEA, 17-OH-Pregnenolone, 17-OH-Progesterone)	503194	82157; 82533; 82626; 83498; 84143	See individual assays	1.5 mL serum Minimum 1.0 mL	Red-top tube only; Do not use gel-barrier tube	Refrigerated (same day) or **frozen**	3-6 days
Congenital Adrenal Hyperplasia (CAH) Test Group III Adult (Late-onset) (Androstenedione, Cortisol, Specific S, DHEA, 17-OH-Pregnenolone, 17-OH-Progesterone)	501072	82157; 82533; 82626; 82634; 83498; 84143	See individual assays	3 mL Serum	Red-top tube only; Do not use gel-barrier tube	Refrigerated (same day) or **frozen**	2-5 days
Corticosteroid Binding Globulin (CBG)	500130	84449	RIA	1 mL Serum Minimum 0.5 mL	Red-top, or gel-barrier tube	Refrigerated (same day) or **frozen**	6-10 days
Corticosterone	500135	82528	HPLC/MS-MS	2 mL Serum Minimum: 0.2 mL	Red-top, or gel-barrier tube	Ambient (same day) or **frozen**	5-8 days
Cortisol (Dexamethasone Suppression Test) With Reflex to Dexamethasone (If the cortisol result is >1.7 µg/dL, dexamethasone test will be performed. Adequate dexamethasone drug level is confirmed with dexamethasone reflex measurement.)	503990	82533; if reflexed, add 80299	HPLC/MS-MS	3 mL Serum or plasma Minimum: 1.0 mL	Red-top, gel-barrier, lavender-top (EDTA), or green-top (heparin) tube	Refrigerated or **frozen**	6-15 days
Cortisol, Free, Equilibrium Dialysis and LC/MS-MS	504020	82530	Equilibrium dialysis, HPLC/MS-MS	1.6 mL Serum Minimum: 0.8 mL	Red-top, or gel-barrier tube	Refrigerated or **frozen**	6-10 days
Cortisol, Free, Serum, With CBG (Free Cortisol, Serum Cortisol, CBG)	500440	82533, 84449	HPLC/MS-MS, RIA, calculation of free cortisol	1 mL Serum Minimum: 0.3 mL	Red-top, or gel-barrier tube	Refrigerated (same day) or **frozen**	6-12 days

Test Name	Test No.	CPT Code(s)	Methodology	Specimen	Container	Storage	Result TAT
Cortisol, Free, Urine (Includes Creatinine)	500410	82530; 82570	HPLC/MS-MS, colorometric	50 mL aliquot Urine (24-hour, well-mixed) Minimum: 5 mL aliquot	Urine container, 24-hour	Refrigerated or **frozen**	6-12 days
Cortisol, Serum or Plasma, Mass Spectrometry	500154	82533	HPLC/MS-MS	0.5 mL Serum or plasma Minimum: 0.1 mL	Red-top, gel-barrier, lavender-top (EDTA) tube, or green-top (heparin) tube	Refrigerated or **frozen**	4-10 days
Cortisol:Cortisone Ratio Profile (Cortisol, Cortisone, Ratio)	503715	82533; 82542	HPLC/MS-MS	1 mL Serum (preferred) or plasma Minimum: 0.3 mL	Red-top, gel-barrier, lavender-top (EDTA), or green-top (heparin) tube	Refrigerated or **frozen** (preferred)	6-10 days
Cortisone, Serum, LCMS	503725	82542	HPLC/MS-MS	0.5 mL Serum (preferred) or plasma Minimum: 0.2 mL	Red-top, gel-barrier, lavender-top (EDTA), or green-top (heparin) tube	Refrigerated or **frozen** (preferred)	4-10 days
C-Peptide, Ultrasensitive	503830	84681	ECL	1 mL Serum or EDTA plasma Minimum: 0.3 mL	Red-top, gel-barrier, or lavender-top (EDTA) tube	Refrigerated or **frozen** (preferred)	6-12 days
Creatinine	001370	82565	Jaffe reaction, colorimetric	1 mL Serum or heparin plasma Minimum: 0.5 mL	Red-top, gel-barrier, or green-top (heparin) tube	Ambient	1 day
C-Telopeptide, Serum	500089	82523	ECLIA	0.5 mL Serum Minimum: 0.3 mL	Red-top, or gel-barrier tube	Refrigerated or **frozen**	3-5 days
Dehydroepiandrosterone (DHEA)	500156	82626	HPLC/MS-MS	0.5 mL Serum Minimum: 0.3 mL	Red-top, or gel-barrier tube	Refrigerated or **frozen**	6-12 days
Dehydroepiandrosterone Sulfate (DHEA-S), Mass Spectrometry	500161	82627	HPLC/MS-MS	0.2 mL Serum Minimum: 0.1 mL	Red-top, or gel-barrier tube	Ambient, refrigerated or **frozen**	5-10 days
Deoxycorticosterone (DOC)	500138	82633	HPLC/MS-MS	3 mL Serum Minimum: 0.5 mL	Red-top, gel-barrier, lavender-top (EDTA), or green-top (heparin) tube	Ambient (same day) or **frozen**	6-15 days
Desoxycortisol, 11-, (Compound S for Metyrapone test)	500550	82634	HPLC/MS-MS	0.5 mL Serum Minimum: 0.2 mL	Red-top, or gel-barrier tube	Refrigerated (same day) or **frozen**	6-10 days
Desoxycortisol, 11-, (Specific Compound S)	500171	82634	HPLC/MS-MS	1 mL Serum (preferred) or plasma Minimum: 0.2 mL	Red-top, gel-barrier, lavender-top (EDTA), or green-top (heparin) tube	Refrigerated (same day) or **frozen**	6-15 days
Dexamethasone, Mass Spectrometry	500118	80299	HPLC/MS-MS	3 mL Serum Minimum: 1 mL	Red-top, gel-barrier, lavender-top (EDTA), or green-top (heparin) tube	Refrigerated or **frozen**	7-13 days

Test Name	Test No.	CPT Code(s)	Methodology	Specimen	Container	Storage	Result TAT
Diabetes Autoimmune Profile (Insulin Antibodies, GAD-65 Antibodies, ICA512 Antibodies, ZNT8 Antibodies)	504050	86337, 86341 (x3)	See individual assays	2.5 mL Serum Minimum: 1 mL	Red-top, or gel-barrier tube	Refrigerated (same day) or **frozen**	8-15 days
Dihydrotestosterone (DHT), Free, LC/MS/Dialysis	504026	84999	Equilibrium dialysis, HPLC/MS-MS	3 mL Serum (preferred) or plasma Minimum: 1.2 mL	Red-top, gel-barrier, or green-top (heparin) tube	Ambient, refrigerated or **frozen** (preferred)	9-15 days
Dihydrotestosterone (DHT), Mass Spectrometry	500142	82642	HPLC/MS-MS	1 mL Serum Minimum: 0.5 mL	Red-top, gel-barrier, lavender-top (EDTA), or green-top (heparin) tube	Ambient, refrigerated or **frozen**	5-10 days
Dopamine, Urine (Includes Creatinine)	500733	82384; 82570	HPLC/MS-MS	50 mL aliquot Urine (24-hour, well-mixed) Minimum: 5 mL aliquot	Urine container, 24-hour	Refrigerated or **frozen** (preferred)	2-7 days
Estradiol, Free (Free Estradiol, Total Estradiol)	500649	82670; 84999	Equilibrium dialysis, HPLC/MS-MS	3 mL Serum Minimum: 1 mL	Red-top, or gel-barrier tube	Ambient (same day) or **frozen**	9-12 days
Estradiol, Free, with SHBG (Free Estradiol, Total Estradiol, SHBG)	500430	82670; 84999; 84270	See individual assays	3 mL Serum Minimum: 1 mL	Red-top, or gel-barrier tube	Ambient (same day) or **frozen**	8-12 days
Estradiol, Serum	500108	82670	HPLC/MS-MS	3 mL Serum Minimum: 1.5 mL	Gel-barrier, lavender-top (EDTA), or green-top (heparin) tube	Ambient or **frozen**	6-10 days
Estriol, Unconjugated (Free)	502684	82677	ICMA	1 mL Serum Minimum: 0.5 mL	Gel-barrier tube	Ambient (same day) or **frozen**	1-3 days
Estrogens, Total	504796	82672	RIA after selective solvent extraction	3 mL Serum (preferred) or plasma Minimum: 1 mL	Gel-barrier, lavender-top (EDTA), or green-top (heparin) tube	Ambient or **frozen**	3-6 days
Estrone, Serum	500634	82679	HPLC/MS-MS	3 mL Serum Minimum: 1 mL	Red-top, gel-barrier, lavender-top (EDTA), or green-top (heparin) tube	Ambient or **frozen**	6-13 days
Ferritin, Serum	501949	82728	ELISA	1 mL Serum or heparin plasma Minimum: 0.2 mL	Red-top, gel-barrier, or green-top (heparin) tube	Ambient	3-5 days
Follicle Stimulating Hormone (FSH)	502280	83001	ECL	1 mL Serum Minimum: 0.2 mL	Red-top, gel-barrier, or lavender-top (EDTA) tube	Refrigerated or **frozen**	5-10 days
Fructosamine	501803	82985	Colorimetric	1 mL Serum or plasma Minimum: 0.5 mL	Red-top, gel-barrier, lavender-top (EDTA), or green-top (heparin) tube	Ambient, refrigerated or **frozen**	2-3 days

Test Name	Test No.	CPT Code(s)	Methodology	Specimen	Container	Storage	Result TAT
Glucose, Plasma	500745	82947	Enzymatic, hexokinase	1 mL Sodium fluoride plasma or EDTA plasma Minimum: 0.3 mL	Gray-top (sodium flouride), lavender-top (EDTA), or green-top (heparin) tube	Ambient, refrigerated or **frozen**	1-3 days
Glucose, Serum	501884	82947	Enzymatic, hexokinase	1 mL Serum Minimum: 0.03 mL	Red-top, gel-barrier, lavender-top (EDTA), or green-top (heparin) tube	Ambient	1-3 days
Glutamic Acid Decarboxylase (GAD-65) Autoantibodies	500611	86341	Immunoprecipitation assay	0.8 mL Serum Minimum: 0.5 mL	Red-top, or gel-barrier tube	Refrigerated or **frozen**	6-12 days
Glycomark®	500115	84378	Enzymatic, colorimetric assay. GlycoMark® is a registered trademark of GlycoMark Inc, New York, NY.	1 mL Serum or plasma Minimum: 0.75 mL	Red-top, gel-barrier, or lavender-top (EDTA) tube	Ambient or refrigerated	3-5 days
Gonadotropin Releasing Hormone (GNRH), Mass Spectrometry	502421	83727	HPLC/MS-MS	1 mL Plasma with aprotinin (Trasylol®) Minimum: 0.5 mL	Lavender-top (EDTA) tube, chilled	**Frozen**	8-12 days
Growth Assessment Test Group V (IGF-1,IGFBP-3)	501007	83520; 84305	See individual assays	1 mL Serum Minimum: 0.2 mL	Red-top, or gel-barrier tube	Refrigerated (same day) or **frozen**	6-8 days
Growth Assessment Test Group VI (IGF-1, IGF-2,IGFBP-3)	501683	83519; 83520; 84305	See individual assays	1.2 mL Serum Minimum: 0.6 mL	Red-top, or gel-barrier tube	Refrigerated (same day) or **frozen**	3-10 days
Growth Hormone Antibodies	500144	86277	Immunoprecipitation assay	1 mL Serum Minimum: 0.5 mL	Red-top, or gel-barrier tube	**Frozen**	7-10 days
Growth Hormone Binding Protein (GHBP)	504591	83520	Ligand-binding immunoprecipitation assay	0.3 mL Serum Minimum: 0.1 mL	Red-top, or gel-barrier tube	Ambient, refrigerated or **frozen**	3-16 days
Growth Hormone, ICMA	500647	83003	ICMA	0.8 mL Serum Minimum: 0.3 mL	Red-top, or gel-barrier tube	Ambient, refrigerated or **frozen**	3-5 days
Growth Hormone, RIA	500632	83003	Double-antibody RIA	1 mL Serum Minimum: 0.4 mL	Red-top, or gel-barrier tube	Ambient, refrigerated or **frozen**	5-10 days
Growth Receptor Test Group I (Growth Hormone, IGF-1, IGFBP-3, GH Binding Protein)	501220	83003; 83519; 83520; 84305	See individual assays	1 mL Serum Minimum: 0.7 mL	Red-top, or gel-barrier tube	Refrigerated (same day) or **frozen**	3-16 days
Growth Receptor Test Group II (Growth Hormone, IGF-1, IGF-2, IGFBP-3, GH Binding Protein)	503322	83003; 83519(x2); 83520; 84305	See individual assays	1.5 mL Serum Minimum: 1 mL	Red-top, or gel-barrier tube	Refrigerated (same day) or **frozen**	3-16 days
Hemoglobin (Hgb) A1C with GlycoMark® Reflex (Hemoglobin A1c, Estimated Average Glucose, GlycoMark® (if reflexed))	503205	83036 If reflexed, add 84378	See individual assays	1 mL serum or plasma and 3 mL whole blood Minimum: 0.75 mL serum or plasma and 1 mL whole blood	Lavender-top (EDTA) whole blood and Red-top, or gel-barrier serum, or lavender-top (EDTA) plasma	Ambient or refrigerated	3-10 days

Test Name	Test No.	CPT Code(s)	Methodology	Specimen	Container	Storage	Result TAT
Hemoglobin A1c With Estimated Average Glucose (eAG) (Hemoglobin A1c, Estimated Average Glucose)	501270	83036	Affinity chromatography HPLC	0.5 mL Whole blood or packed cells Minimum: 0.2 mL	Lavender-top (EDTA) tube	Ambient, refrigerated or **frozen**	3-5 days
Hirsutism Test Group I (Dehydroepiandrosterone-sulfate (DHEA-S), Testosterone, Free Testosterone, SHBG)	500066	82627; 84270; 84402; 84403	See individual assays	3 mL Serum Minimum: 1.5 mL	Red-top, or gel-barrier tube	Refrigerated (same day) or **frozen**	6-8 days
Hirsutism Test Group II (Androstenedione, Dehydroepiandrosterone-sulfate (DHEA-S), Testosterone, Free Testosterone, SHBG)	502236	82157; 82627; 84270; 84402; 84403	See individual assays	2 mL Serum Minimum: 1.1 mL	Red-top, or gel-barrier tube	Refrigerated (same day) or **frozen**	3-5 days
Hirsutism Test Group IV (Androstanediol Glucuronide, Androstenedione, Dehydroepiandrosterone-sulfate (DHEA-S), Testosterone, Free Testosterone, SHBG)	501663	82154; 82157; 82627; 84270; 84402; 84403	See individual assays	3.2 mL Serum Minimum: 1.7 mL	Red-top tube only; Do not use gel-barrier tube	Refrigerated (same day) or **frozen**	3-12 days
Homovanillic Acid (HVA), Urine (Includes Creatinine)	501814	82570; 83150	HPLC/MS-MS, Jaffe reaction, colorimetric	50 mL aliquot Urine (24-hour, well-mixed) Minimum: 10 mL aliquot	Urine container, 24-hour	Refrigerated	2-7 days
Hydroxycorticosterone, 18-, Mass Spectrometry	500778	82542	HPLC/MS-MS	3 mL Serum Minimum: 1 mL	Red-top, gel-barrier, lavender-top (EDTA), or green-top (heparin) tube	Refrigerated (same day) or **frozen**	7-12 days
Hydroxyindoleacetic Acid 5- (5-HIAA), plasma	504510	83497	HPLC/MS-MS	1.0 mL Plasma Minimum: 0.5 mL	Green-top (heparin), or lavender-top (EDTA) tube	Ambient, refrigerated or **frozen**	8-15 days
Hydroxyindoleacetic Acid, 5- (5-HIAA), Urine (Includes Creatinine)	500720	82570; 83497	HPLC/MS-MS, Jaffe reaction, colorimetric	50 mL aliquot. Acidify urine to pH 4.0 with acetic acid. Do not use strong mineral acid! Urine (24-hour, well-mixed) Minimum: 5 mL aliquot	Urine container, 24-hour, with acetic acid	Refrigerated	2-16 days
Hydroxypregnenolone, 17-	140715	84143	HPLC/MS-MS	1 mL Serum Minimum: 0.1 mL	Red-top, gel-barrier, or lavender-top (EDTA) tube	Refrigerated (same day) or **frozen**	5-10 days
Hydroxyprogesterone, 17a-, (17-OHP)	500163	83498	HPLC/MS-MS	1 mL Serum Minimum: 0.1 mL	Red-top, gel-barrier, lavender-top (EDTA), or green-top (heparin) tube	Ambient, refrigerated or **frozen**	4-10 days
Hypoglycemia Test Group I (Cortisol, Glucose, Growth Hormone, Insulin)	501106	82533; 82947; 83003; 83525	See individual assays	2 mL Serum Minimum: 1 mL	Red-top, or gel-barrier tube	**Frozen**	2-4 days

Test Name	Test No.	CPT Code(s)	Methodology	Specimen	Container	Storage	Result TAT
IA-2/ICA-512 Autoantibodies	141531	86341	ELISA	1 mL Serum Minimum: 0.5 mL	Red-top, or gel-barrier tube	Refrigerated or **frozen**	5-10 days
Insulin	503068	83525	Immunometric assay (IMA)	0.5 mL Serum or EDTA plasma Minimum: 0.2 mL	Red-top, gel-barrier, or lavender-top (EDTA) tube	**Frozen**	3-10 days
Insulin Antibodies	141598	86337	Insulin-I125 binding capacity	0.5 mL Serum Minimum: 0.2 mL	Red-top, or gel-barrier tube	Refrigerated or **frozen**	7-12 days
Insulin, Free and Total (Insulin, Free; Insulin, Total)	501561	83525; 83527	Immunometric assay (IMA)	3 mL Serum Minimum: 1.5 mL	Red-top, or gel-barrier tube	Refrigerated	5-12 days
Insulin-like Growth Factor 1 (IGF-1)	500485	84305	Blocking RIA after acid:alcohol extraction	0.5 mL Serum Minimum: 0.1 mL	Red-top, or gel-barrier tube	Ambient (same day) or **frozen**	6-10 days
Insulin-Like Growth Factor 1 (IGF-1), Pediatric with Z Score	503660	84305	Blocking RIA after acid:alcohol extraction	0.5 mL Serum Minimum: 0.1 mL	Red-top, or gel-barrier tube	Ambient (same day) or **frozen**	6-12 days
Insulin-like Growth Factor 2 (IGF-2)	141770	83519	Blocking RIA after acid:alcohol extraction	0.5 mL Serum Minimum: 0.1 mL	Red-top, or gel-barrier tube	Refrigerated (same day) or **frozen**	9-13 days
Insulin-like Growth Factor-binding Protein 1 (IGFBP-1)	140822	83520	ICMA	0.5 mL Serum Minimum: 0.1 mL	Red-top, or gel-barrier tube	Refrigerated (same day) or **frozen**	6-12 days
Insulin-like Growth Factor-binding Protein 2 (IGFBP-2)	500133	83519	ECL	1 mL Serum or plasma Minimum: 0.3 mL	Red-top, gel-barrier, or lavender-top (EDTA) tube	Refrigerated (same day) or **frozen**	5-10 days
Insulin-like Growth Factor-binding Protein 3 (IGFBP-3)	500644	83520	RIA in dilute serum	1 mL Serum or plasma Minimum: 0.25 mL	Red-top, gel-barrier, or lavender-top (EDTA) tube	Ambient or **frozen**	3-6 days
IPTH Parathyroid Aspirate	503000	83970	ICMA	1 mL Parathyroid aspirate	Lymph Node and Parathyroid aspirate kit (peoplesoft # 75203)	**Frozen**	2-4 days
Iron	501807	83540	Ferrozene	1 mL Serum or plasma Minimum: 0.5 mL	Red-top, gel-barrier, or green-top (heparin) tube	Ambient	3-5 days
Islet Cell Dysfunction Test Group 1 (Insulin, Proinsulin, Proinsulin:Insulin Ratio)	500757	83525; 84206	See individual assays	1 mL EDTA plasma or serum Minimum: 0.6 mL	Red-top, gel-barrier, lavender-top (EDTA), or green-top (heparin) tube	**Frozen**	7-15 days
Islet Cell Dysfunction Test Group 2 (Glucose, Insulin, Proinsulin, Proinsulin:Insulin Ratio)	500759	82947; 83525; 84206	See individual assays	1.5 mL EDTA plasma or serum Minimum: 1 mL	Red-top, gel-barrier, lavender-top (EDTA), or green-top (heparin) tube	**Frozen**	2-6 days
Leptin	500716	83520	ELISA	1 mL Serum or plasma Minimum: 0.5 mL	Red-top, gel-barrier, lavender-top (EDTA), or green-top (heparin) tube	Refrigerated (same day) or **frozen**	11-15 days

Test Name	Test No.	CPT Code(s)	Methodology	Specimen	Container	Storage	Result TAT
Lipid Panel (Cholesterol, High-density Lipoprotein (HDL) Cholesterol,Triglycerides)	501856	80061	See individual assays	5 mL Serum Minimum: 2 mL	Red-top, gel-barrier, or lavender-top (EDTA) tube	Ambient	3-5 days
Luteinizing Hormone (LH)	502286	83002	ECL	1 mL Serum Minimum: 0.2 mL	Red-top, gel-barrier, or lavender-top (EDTA) tube	Refrigerated (same day) or **frozen**	5-10 days
Macroprolactin		84146(x2)	ECL	1 mL Serum Minimum: 0.2 mL	Red-top, gel-barrier, or lavender-top (EDTA) tube	Refrigerated (same day) or **frozen**	5-10 days
Maturity-Onset Diabetes of the Young (MODY) Genetic Profile	504603	81404; 81405(x2); 81406(x2)	Sanger sequencing and MLPA	3 mL Serum Minimum: 1 mL	Lavender-top (EDTA) tube	Ambient; do not freeze	14-42 days
***MEN1* Gene, Sequencing Analysis**	504010	81405	Polymerase chain reaction (PCR) of targeted /MEN1/ gene exons, DNA sequencing of those PCR products	3 mL Whole blood Minimum: 1 mL	Lavender-top (EDTA) tube, or yellow-top (ACD) tube	Ambient; do not freeze	14-28 days
MEN2: *RET* Gene, Sequencing Analysis	504008	81405	Polymerase chain reaction (PCR) of targeted *RET* gene exons, DNA sequencing of those PCR products	3 mL Whole blood Minimum: 1 mL	Lavender-top (EDTA) tube, or yellow-top (ACD) tube	Ambient; do not freeze	14-21 days
Menopausal Profile (Postmenopausal) (Estradiol, Follicle-stimulating Hormone (FSH), Luteinizing Hormone (LH), Progesterone)	501218	82670; 83001; 83002; 84144	See individual assays	5 mL Serum Minimum: 2 mL	Red-top, or gel-barrier tube	Refrigerated (same day) or **frozen**	6-10 days
Menopausal Test Group (Postmenopausal) (Estradiol, Follicle-stimulating Hormone (FSH), Luteinizing Hormone (LH))	500888	82670; 83001; 83002	See individual assays	3 mL Serum	Red-top, gel-barrier, or lavender-top (EDTA) tube	Refrigerated (same day) or **frozen**	4-7 days
Metanephrines, Fractionated Urine (Includes Creatinine) (Metanephrine, Normetanephrine, Creatinine)	501533	83835; 82570	HPLC/MS-MS, colormetric	10 mL aliquot Urine (24-hour, well-mixed) Minimum: 1 mL aliquot	Urine container, 24-hour	Ambient, refrigerated or **frozen**	7-15 days
Metanephrines, Total, Urine (Includes Creatinine)	500480	83835; 82570	HPLC/MS-MS, colorometric	10 mL aliquot Urine (24-hour, well-mixed) Minimum: 1 mL aliquot	Urine container, 24-hour	Ambient, refrigerated or **frozen**	7-15 days
Albumin, Urine (Includes Creatinine)	500870	82043; 82570	Immunoturbidimetric, colorometric	30 mL aliquot Urine (24-hour or overnight) Minimum: 1 mL aliquot	Urine container, 24-hour	Ambient or refrigerated	4-5 days
Mineralocorticoid Adult Test Group (Aldosterone, Corticosterone, 18-OH-Corticosterone, Deoxycorticosterone)	503196	82088; 82528; 82542; 82633	See individual assays	4 mL Serum Minimum: 2.5 mL	Red-top, or gel-barrier tube	Refrigerated (same day) or **frozen**	3-9 days

Test Name	Test No.	CPT Code(s)	Methodology	Specimen	Container	Storage	Result TAT
Mineralocorticoid Pediatric Profile (Aldosterone, Corticosterone, 18-OH-Corticosterone, Deoxycorticosterone)	500285	82088; 82528; 82542; 82633	See individual assays	2.5 mL Serum Minimum: 1.5 mL	Red-top, gel-barrier, lavender-top (EDTA), or green-top (heparin) tube	Refrigerated (same day) or **frozen**	6-12 days
Parathyroid Hormone, Intact (IPTH, Includes Calcium) (Intact Parathyroid Hormone, Calcium)	500692	82310; 83970	ICMA, ion-specific electrode	2 mL plasma and 1 mL serum Serum and plasma Minimum: 0.5 mL plasma and 0.5 mL serum	Lavender-top (EDTA) **and** red-top tube	Ambient, refrigerated or **frozen**	3-10 days
Parathyroid Hormone-Related Peptide (PTHrP)	503380	82397	ECL	1 mL EDTA plasma or plasma with aprotinin (Trasylol®). (Trasylol acceptable, not required.) Minimum: 0.5 mL	Lavender-top (EDTA) tube	**Frozen**	5-10 days
Pregnenolone	140707	84140	HPLC/MS-MS	2.5 mL Serum Minimum: 1.1 mL	Red-top, gel-barrier, lavender-top (EDTA), or green-top (heparin) tube	Refrigerated (same day) or **frozen**	7-10 days
Premature Adrenarche Profile I (Androstenedione, DHEA-S, 17-OH-Progesterone, Testosterone)	500767	82157; 82627; 83498; 84403	See individual assays	2 mL Serum Minimum: 1.5 mL	Red-top tube only; Do not use gel-barrier tube	Refrigerated (same day) or **frozen**	6-15 days
Premature Adrenarche Profile II (Androstenedione, DHEA, 17-OH-Pregnenolone, 17-OH-Progesterone, Testosterone)	500913	82157; 82626; 83498; 84143; 84403	See individual assays	2 mL Serum Minimum: 1.5 mL	Red-top, lavender-top (EDTA), or green-top (heparin) tube; do not use gel-barrier tube	Refrigerated (same day) or **frozen**	7-10 days
Progesterone, Free with Total Progesterone (Total Progesterone, Free Progesterone)	503658	84144; 84999	HPLC/MS-MS	1.6 mL Serum (preferred) or EDTA plasma Minimum: 0.8 mL	Red-top, gel-barrier, lavender-top (EDTA), or green-top (heparin) tube	Refrigerated or **frozen** (preferred)	8-12 days
Progesterone, Mass Spectrometry	500167	84144	HPLC/MS-MS	1 mL Serum Minimum: 0.3 mL	Red-top, gel-barrier, lavender-top (EDTA), or green-top (heparin) tube	Ambient (same day) or **frozen**	5-10 days
Proinsulin	500722	84206	ICMA	1 mL Plasma or serum Minimum: 0.5 mL	Red-top, gel-barrier, lavender-top (EDTA), or green-top (heparin) tube	Refrigerated (same day) or **frozen**	5-9 days
Prolactin With Dilution for Hook Effect	500557	84146	ICMA	1 mL Serum Minimum: 0.1 mL	Red-top, gel-barrier, or lavender-top (EDTA) tube	Refrigerated (same day) or **frozen**	5-10 days

Test Name	Test No.	CPT Code(s)	Methodology	Specimen	Container	Storage	Result TAT
Renin, Plasma (Plasma Renin Activity)	500458	84244	RIA of angiotensin I generated at pH 5.5 at multiple time points	2 mL EDTA plasma only. Collect 4 mL of blood in lavender-top venipuncture tube. Mix gently by inversion. Centrifuge immediately after collection. Minimum: 0.4 mL	Lavender-top (EDTA) tube	**Frozen**	5-10 days
Resin T3 Uptake, Serum	500850	84479	ICMA, calculation	1 mL Serum Minimum: 0.1 mL	Gel-barrier, or red-top tube	Refrigerated (same day) or **frozen**	3-10 days
Salivary Cortisol, LC/MS-MS	500179	82533	HPLC/MS-MS	1 mL Saliva Minimum: 1 mL	Saliva Collection Device. Salivary Hormone Collection Kits are available (Peoplesoft # 98572)	Ambient or refrigerated	6-10 days
Sex Hormone-binding Globulin (SHBG), Serum	500848	84270	ECLIA	0.5 mL Serum Minimum: 0.3 mL	Red-top, or gel-barrier tube	Refrigerated or **frozen** (preferred)	3-5 days
***SHOX,* DHPLC**	500110	81479	Mutation analysis by polymerase chain reaction (PCR), denaturing high-pressure liquid chromatography (DHPLC) and sequencing as needed	3 mL whole blood Minimum: 1 mL	Lavender-top (EDTA), or yellow-top (ACD) tube	Ambient or refrigerated	18-30 days
Testicular Function Complete Testosterone Evaluation Group (Testosterone, Free Testosterone, Bioavailable Testosterone, SHBG)	501247	84402; 84410; 84270	See individual assays	3 mL Serum Minimum: 1.5 mL	Red-top, or gel-barrier tube	Refrigerated (same day) or **frozen**	3-5 days
Testicular Function Group I (Follicle-stimulating Hormone (FSH), Luteinizing Hormone (LH), Testosterone)	501048	83001; 83002; 84403	See individual assays	2 mL Serum Minimum: 1 mL	Red-top, gel-barrier, or lavender-top (EDTA) tube	Refrigerated (same day) or **frozen**	3-5 days
Testicular Function Group III (Estradiol, Follicle-stimulating Hormone (FSH), Luteinizing Hormone (LH), Prolactin, Testosterone)	503450	82670; 83001; 83002; 84146; 84403	See individual assays	3.7 mL Serum Minimum: 2.5 mL	Red-top, or gel-barrier tube	Refrigerated (same day) or **frozen**	4-7 days
Testosterone, Bioavailable, With Sex Hormone-binding Globulin (Testosterone, Bioavailable Testosterone, SHBG)	500650	84270; 84410	See individual assays	2 mL Serum Minimum: 0.6 mL	Red-top, or gel-barrier tube	Refrigerated or **frozen**	6-10 days
Testosterone, Free, With Sex Hormone-binding Globulin (Total Testosterone, Free Testosterone, SHBG)	500102	84270; 84402; 84403	See individual assays	2 mL Serum Minimum: 1 mL	Red-top, or gel-barrier tube	Refrigerated or **frozen**	6-12 days
Testosterone, Total and Free (Total Testosterone, Free Testosterone)	500726	84402; 84403	Equilibrium dialysis, HPLC/MS-MS	2 mL Serum Minimum: 1 mL	Red-top, or gel-barrier tube	Refrigerated or **frozen**	6-10 days

Test Name	Test No.	CPT Code(s)	Methodology	Specimen	Container	Storage	Result TAT
Testosterone, Total, Serum, Mass Spectrometry	500159	84403	HPLC/MS-MS	1 mL Serum Minimum: 0.5 mL	Red-top, gel-barrier, lavender-top (EDTA), or green-top (heparin) tube	Refrigerated or **frozen**	5-11 days
Thyroglobulin (With Antithyroglobulin screen), Comprehensive	500540	84432; 86800	ICMA or double antibody RIA when anti-Tg antibodies are present	5 mL Serum Minimum: 1.5 mL	Red-top, or gel-barrier tube	Ambient or **frozen**	4-10 days
Thyroglobulin, Lymph Node Aspirate	502380	84432	ICMA	1 mL Lymph node aspirate Minimum: 1 mL	Lymph Node and Parathyroid aspirate kit (peoplesoft # 75203)	Refrigerated or **frozen**	3-15 days
Thyroid Function, Adult Hyperthyroid/TSH Suppression Test Group (Thyrioid-stimulating Hormone(TSH)-ICMA, T3, T4, T3 Uptake, Free T4 Index)	500713	84436; 84443; 84479; 84480	See individual assays	2 mL Serum Minimum: 0.8 mL	Red-top, or gel-barrier tube	Refrigerated or **frozen**	1-3 days
Thyroid Function, Adult Hypothyroid Test Group (Thyroid-stimulating Hormone (TSH)-ICMA, T4, T3 Uptake, Free T4 Index)	500490	84436; 84443; 84479	See individual assays	1.5 mL Serum Minimum: 0.7 mL	Red-top, or gel-barrier tube	**Frozen**	4-15 days
Thyroid Function, Adult Special Thyroid Test Group (Thyroid-stimulating Hormone (TSH)-ICMA, T3, T4)	500559	84436; 84443; 84480	See individual assays	2 mL Serum Minimum: 0.7 mL	Red-top, gel-barrier, lavender-top (EDTA), or green-top (heparin) tube	Refrigerated or **frozen**	3-10 days
Thyroid Function, Adult Thyroid Test Group (T4, T3 Uptake, Free T4 Index)	500712	84436; 84479	See individual assays	1 mL Serum Minimum: 0.2 mL	Red-top, or gel-barrier tube	Refrigerated or **frozen**	6-15 days
Thyroid Function, Pediatric Thyroid Autoantibodies Test Group (Thyroglobulin Antibodies, Thyroid Peroxidase Antibodies)	501015	86376; 86800	See individual assays	1 mL Serum Minimum: 0.5 mL	Red-top, or gel-barrier tube	**Frozen**	3-10 days
Thyroid Peroxidase Antibodies (Anti-TPO)	500638	86376	Chemiluminescence	0.5 mL Serum Minimum: 0.3 mL	Gel-barrier tube	Refrigerated or **frozen**	3-6 days
Thyroid Stimulating Hormone (TSH), ICMA	500477	84443	ICMA	1 mL Serum Minimum: 0.2 mL	Red-top tube	Refrigerated or **frozen**	3-6 days
Thyroxine (T4), Automated	500348	84436	ICMA	1 mL Serum Minimum: 0.2 mL	Red-top, or gel-barrier tube	Refrigerated or **frozen**	3-5 days
Thyroxine, Free (FT4), Automated Analog Immunoassay	500835	84439	ECLIA	0.8 mL Serum Minimum: 0.3 mL	Red-top, or gel-barrier tube	Refrigerated or **frozen**	3-12 days
Thyroxine, Free, By Dialysis and HPLC Tandem Mass Spectrometry	501902	84439	Direct dialysis, HPLC/MS-MS	1 mL Serum or plasma Minimum: 0.4 mL	Red-top, gel-barrier, or lavender-top (EDTA) tube	Refrigerated or **frozen**	6-11 days
Thyroxine-binding Globulin (TBG)	500724	84442	ICMA	1 mL Serum or EDTA plasma Minimum: 0.3 mL	Red-top, gel-barrier, or lavender-top (EDTA) tube	**Frozen**	3-10 days

Test Name	Test No.	CPT Code(s)	Methodology	Specimen	Container	Storage	Result TAT
Transferrin Saturation, Serum (Iron, Transferrin)	501819	83540; 84466	Ferrachrome, turbidimetric	1 mL Serum Minimum: 0.5 mL	Red-top, or gel-barrier tube	Refrigerated or **frozen**	3-5 days
Triiodothyronine (T3)	500563	84480	ICMA	2 mL Serum Minimum: 0.2 mL	Red-top tube	Refrigerated or **frozen**	3-5 days
Triiodothyronine, Free (Includes T3) (Free T3, T3)	500355	84480; 84481	See individual assays	1 mL Serum Minimum: 0.3 mL	Red-top tube	Refrigerated or **frozen**	1-3 days
Triiodothyronine, Free Only	500834	84481	ECLIA	0.5 mL Serum Minimum: 0.2 mL	Red-top, or gel-barrier tube	Refrigerated or **frozen**	3-5 days
Triiodothyronine, Free, by Dialysis and Mass Spectrometry	503600	84481	Equilibrium dialysis, HPLC/MS-MS	1 mL Serum or EDTA plasma Minimum: 0.3 mL	Red-top, gel-barrier, lavender-top (EDTA), or green-top (heparin) tube	Refrigerated or **frozen** (preferred)	6-10 days
Triiodothyronine, Reverse	503663	84482	HPLC/MS-MS	1 mL Serum or EDTA plasma Minimum: 0.5 mL	Gel-barrier, lavender-top (EDTA), or green-top (heparin) tube	Refrigerated or **frozen**	3-9 days
TSH Receptor Antibody (TRAb/TBII)	500538	83519	Binding inhibition assay	1 mL Serum Minimum: 0.3 mL	Red-top, or gel-barrier tube	Refrigerated or **frozen**	6-13 days
Vanillylmandelic Acid (VMA) (Includes Creatinine)	500496	82570; 84585	HPLC/MS-MS, Jaffe reaction, colorimetric	50 mL aliquot Urine (24-hour, well mixed) Minimum: 1 mL aliquot	Urine container, 24-hour, with 6N HCl	Refrigerated	2-7 days
Vitamin D, 1, 25-Dihydroxy	500600	82652	HPLC/MS-MS	2 mL Serum or plasma Minimum: 0.8 mL	Red-top, gel-barrier, lavender-top (EDTA), or green-top (heparin) tube	Refrigerated (same day) or **frozen**	5-10 days
Vitamin D, 25-Hydroxy, Fractionated, Mass Spectrometry (Total Vitamin D, Vitamin D2, Vitamin D3)	504115	82306	HPLC/MS-MS	1.0 mL Serum (preferred) or plasma Minimum: 0.5 mL	Red-top, gel-barrier, lavender-top (EDTA), or green-top (heparin) tube	Refrigerated or **frozen**	4-10 days
Vitamin D, 25-Hydroxy, Total, Mass Spectrometry	500510	82306	HPLC/MS-MS	1.5 mL Serum Minimum: 0.5 mL	Red-top, gel-barrier, lavender-top (EDTA), or green-top (heparin) tube	Refrigerated or **frozen**	6-10 days
ZnT8 Antibodies	503995	86341	ELISA	0.5 mL Serum (preferred) or plasma (heparin) Minimum: 0.2 mL	Red-top tube, gel-barrier tube, or green-top (heparin) tube	Refrigerated or **frozen**	6-10 days