PAEDIATRIC ENDOCRINOLOGY PROTOCOLS

Department of Clinical Biochemistry, Royal Infirmary of Edinburgh. 51 Little France Crescent, EH16 4SA. General Enquiries: 0131 242 6806 (Ext: 26806). Endocrine Biochemist: 0131 242 6880 (Ext: 26880)

Introduction

Samples collected at timed intervals before and after the administration of test compounds or in special conditions are occasionally used. These tests must be carefully performed, as relatively minor deviations from the standard procedures can invalidate the test or make result interpretation difficult. The need to repeat a test because of poor technique is unfair to all concerned, and is particularly hard on the patient.
Please use the special request forms that are available for the most commonly requested tests (GnRH, Synacthen, Insulin tolerance, Glucose tolerance). These give timings and simplify sample handling.

The laboratory holds protocols for a number of other dynamic function tests that are rarely carried out. Contact the Duty Biochemist for advice.

Glucose Tolerance Test

The 2 hour standard oral glucose tolerance test may be used to establish a diagnosis of Diabetes Mellitus.

It is unnecessary if a child has severe symptoms of diabetes and either a random venous plasma laboratory glucose concentration of 11.1 mmol/L or higher, or a fasting concentration of 7.0 mmol/L or higher. [Definition, Diagnosis and Classification of Diabetes Mellitus. WHO criteria 1999].
Glucose is measured at 0min and 120 min only.
The glucose tolerance test is not recommended to assess insulin resistance in obese children.

Procedure

1. Do not perform glucose tolerance tests on patients known to be suffering from an infection, patients with uncontrolled thyroid dysfunction, or patients recovering from severe stress (e.g. surgery) as these alter insulin sensitivity.
2. Ensure that the child has had an adequate diet (minimum of 150g/day of carbohydrate) for at least 5 days before the test. Fast the patient overnight (4 hours for infants) but avoid more prolonged fasting. Drinks of water (no sweet drinks) are allowed during this period.
3. The next morning, take a venous blood sample for laboratory glucose
4. Give the patient glucose monohydrate by mouth according to body weight, 1.925 grams per kg body weight, (not to exceed 82.5 grams - equivalent to 75grams anhydrous glucose, the standard adult dose). Dissolve the dose in 100-200 mL chilled water, flavoured with lemon. The drink should be fully consumed in 5-10 minutes.
5. Collect one further venous blood sample for laboratory glucose 120min after the dose.

Interpretation

Glucose results (mmol/L) are interpreted as follows:

	Fasting		2 hours post glucose
Diabetes mellitus	7.0 or more	or	11.1 or more
Impaired glucose tolerance	Less than 7.0	and	7.8 – 11.1
Impaired fasting glycaemia	6.1 – 6.9	and	Less than 7.8
Normal results	Less than 5.6	and	Less than 7.8

In patients without symptoms diagnosis should not be based on a single glucose determination. At least one additional glucose test result with a value in the diabetic range is required, either fasting, random or 2 hours after a standard glucose load.
[Diagnosis and Classification of Diabetes Mellitus. WHO criteria 1999,
Expert Committee on the Diagnosis and Classification of Diabetes Mellitus 2003].

Gonadotrophin Releasing Hormone (GnRH) Test

Gonadotrophin releasing hormone (GnRH) is an oligopeptide hormone secreted by the hypothalamus, which controls the release of luteinising hormone (LH) and follicle stimulating hormone (FSH) by the anterior pituitary gland. In older children and adults, loss of the LH and FSH response to GnRH may be an early indication of anterior pituitary disease. The test is of no value in patients with elevated LH/FSH levels, indicating primary gonadal failure. [Managed Clinical Network of Scottish Paediatric Endocrine Group. Dynamic Function Test Handbook for Clinicians. Jan 11].
This test may be combined with the Insulin Hypoglycaemia Test; increase volumes of blood collected accordingly. Use an Endocrine Function Test Request Form (available from lab) to accompany all samples for this test. Label all tubes as usual with the patient's surname, first name, date of birth, date and time, PLUS the sample time (0 min, 20 min, 60 min). Avoid HCG injections during the test, as cross-reaction in LH analyses may falsely elevate results.

Procedure

1. No special preparation of the patient is necessary. Collect blood for LH, FSH and oestradiol (girls) or testosterone (boys). Give 100 micrograms of Gonadorelin or LHRH (all ages) by intravenous injection.
2. Take further blood samples for LH and FSH 20 minutes and 60 minutes after the GnRH injection.

Interpretation*

Sex / Pubertal stage	Peak LH (U/L)		Peak FSH (U/L)	
	Median	Ref. range	Median	Ref. Range
Boys Prepubertal	3.0	<13.0	4.0	1.2 – 9.0
G2 - 3	11.0	<22.0	4.5	0.8 – 22.0
G4 - 5	12.5	5.5 – 48.0	3.5	1.0 – 18.0
Girls Prepubertal	2.0	<11.0	8.5	1.0 – 22.0
B2	4.0	<20	9.0	5.0 – 22.0
B3 - 5	17.0	5.5 – 28.0	9.0	6.0 - 22.0

***Values updated October 2021, following change to Roche diagnostics.**

Normal pre-pubertal children usually have baseline LH <1.5 U/L and FSH <4.0 U/L, but may show small increments in response to GnRH, especially in late prepuberty. FSH responses to GnRH change little through puberty. LH response to GnRH increases in early-mid puberty in boys, and in mid-late puberty in girls, but there is overlap between successive pubertal stages.
Central precocious puberty in boys or girls is usually (but not always) associated with an LH response to GnRH to >5 U/L (updated Oct 2021). Girls with isolated *premature thelarche* usually have a lower LH response (<5 U/L). FSH responses are not discriminatory and are usually >4 U/L in both conditions.
Primary gonadal failure in patients older than 10 years is usually associated with increased baseline levels of LH and FSH and an exaggerated response to GnRH.
Boys with *constitutional delay of puberty* have LH responses similar to normal (younger) prepubertal boys. Boys with *hypogonadotrophic hypogonadism* usually have lower responses of LH (typically to <1.0 U/L) and FSH (typically to <3.0 U/L) than either normal prepubertal boys or boys with constitutional delay.

Human Chorionic Gonadotrophin (HCG) Stimulation Test

In the normal male child HCG increases testicular secretion of testosterone. This test examines the capacity of Leydig cells to respond to HCG and secrete testosterone. It is used to detect functioning testicular tissue in some cases of undescended testes. It can also be useful in the differential diagnosis of male hypogonadism, androgen insensitivity, (androgen receptor defects) and inborn errors of androgen synthesis.

Procedure

1. No special preparation of the patient is necessary. On the first day (day 1), collect 3.0 mL blood at 0900 - 1000h for testosterone, dihydrotestosterone and androstenedione and send to the laboratory. Label the request form 'HCG Test Day 1'.
2. At about 1000h (just after the baseline sample has been taken) give HCG by intramuscular injection. HCG (Pregnyl) is available in ampoules containing 5000 IU. For children younger than 14 years, the dose is 1500 IU: dissolve the contents of the ampoule in 1 mL normal saline and give 0.3 mL by intramuscular injection. For children older than 14 years, the dose is 3000 IU: dissolve the contents of the ampoule in 0.5 mL normal saline and give 0.3 mL by intramuscular injection.
3. On the next two days (days 2 and 3) give HCG (children younger than 14 years 1500 IU; adults and children older than 14 years 3000 IU) by intramuscular injection at about 1000h.
4. On the last two days (days 4 and 5) collect blood at 0900-1000h for testosterone dihydrotestosterone and androstenedione. Send each sample to the laboratory (by van or post if the specimens are taken by the child's GP). Label the request forms 'HCG Test Day 4' and 'HCG Test Day 5' respectively.

Interpretation

A normal Leydig cell response to HCG is indicated by an increase of at least 3-fold in plasma testosterone to a peak of >5 nmol/L in boys aged 0 – 14 years, or >10 nmol/L in boys older than 14 years.
For males aged 6m to puberty, a normal unstimulated testosterone:dihydrotestosterone ratio is <20; a normal post-HCG stimulation test ratio is <27. An increased ratio is consistent with 5-a-reductase deficiency.
In males, the testosterone:androstenedione ratio after HCG stimulation is usually greater than 1.0. A ratio less than 0.8 after HCG stimulation is consistent with gonadal 17-beta-hydroxysteroid dehydrogenase 3 deficiency, but may also occur in dysplastic testes or testes with absent Leydig cells.

Insulin Hypoglycaemia Test

This test is potentially dangerous [Shah et al Brit Med J 1992;304:173-4] and should only be done after consultation with a Consultant Paediatric Endocrinologist. No child with a history of epilepsy or cardiac arrhythmias should be subjected to this test.
Any form of stress results in secretion of the hypothalamic hormones, growth hormone releasing hormone (GHRH) and corticotrophin releasing hormone (CRH). These in turn stimulate the release of pituitary growth hormone (GH) and adrenocorticotrophic hormone (ACTH), in the latter case leading to adrenal cortisol secretion. Insulin administration is used to produce stress in the form of hypoglycaemia, and hypothalamic - pituitary - adrenal function is assessed by GH and cortisol responses to the hypoglycaemic stimulus. [Hindmarsh & Swift. Arch Dis Child 1995;72:362-8; Tetlaw and Clayton in: Brook, Clayton, Brown (Eds) Clinical paediatric endocrinology 5th edn 2005. Blackwell Scientific, Oxford p526-527].

If the test is to be done on peri-pubertal children (bone age greater than 10 years, no signs of puberty in girls or testicular volume less than 8mL in boys), discuss with endocrinology the advisability of "priming" with the appropriate sex steroid.

This test is often combined with the Gonadotrophin Releasing Hormone (GnRH) test; the volume of blood collected will need to be increased accordingly. Use an Endocrine Function Test Request Form (available from lab) to accompany all samples for this test. Label all tubes as usual with the patient's surname, first name, date of birth, date and time, PLUS the sample time (-30 min, 0 min, 20 min etc).
This test is designed to produce symptomatic hypoglycaemia (pale, sweaty). If symptoms are more severe (impaired or loss of consciousness) the child must be treated immediately (see below). Continuous observation for the symptoms of severe hypoglycaemia is essential throughout the test, and for half an hour after its completion.

Procedure

1. The patient should be fasted overnight (4 hours for infants); drinks of water are allowed.
2. Before beginning the test, have available a glucose drink: 4 heaped teaspoons (equivalent to approximately 40g) dextrose powder dissolved in approximately half a glass of squash. Ensure that glucose, hydrocortisone and adrenaline are also available for intravenous injection if necessary (see below).
3. Observe the child continuously during the test for symptoms of severe hypoglycaemia, and check the glucose concentration in each blood sample collected using the ward blood glucose meter, or more frequently if the child is developing hypoglycaemic symptoms.
4. If symptoms of severe hypoglycaemia do develop they must be treated immediately by giving intravenous glucose 200 mg per kg body weight (10% dextrose, 2mL per kg) over 3 minutes. If the response is poor, give 100 mg hydrocortisone by intravenous injection. Continue with a glucose infusion i/v at 10 mg per kg per minute (6 millilitres per kilogram per hour of 10% dextrose). Check blood glucose using the ward meter after 5 min and adjust the glucose infusion to maintain a blood glucose of 5-8 mmol/L and no higher. If there is no improvement in conscious level after normal blood glucose is restored, an alternative explanation should be sought. It is not necessary to discontinue the test and, if possible, continue blood sampling.
5. Before beginning the test, weigh the patient and insert an indwelling catheter at least 30 minutes before taking the baseline samples. The patient should be resting throughout the test. Start the test between 0800h and 0900h.
6. Take blood sample for glucose, cortisol and GH determinations. Label this sample "-30 min". Check blood glucose concentration using the ward meter.
7. After 30 minutes take a second baseline blood sample for glucose, cortisol and GH. Label this sample "0 min". Check blood glucose concentration using the ward meter.
8. If blood glucose, measured using the ward meter, is less than 3.5 mmol/L in either of the two baseline samples, do NOT give insulin but continue to take blood samples (11. Below) and record whether child has symptoms (pale, sweating). If blood glucose, measured using the ward meter, is between 3.5 and 4.5 mmol/L in either of the two baseline samples, give half the dose of insulin (see below) and continue the test.
9. The soluble insulin dose is normally 0.15 units per kg body weight, but this should be reduced to 0.075 - 0.1 units per kg in patients who might be unduly sensitive to insulin. These include patients with hypothalamic or pituitary hypofunction, those with severe malnutrition (e.g. due to anorexia nervosa) or those with baseline blood glucose between 3.5 and 4.5 mmol/L (see (8) above). The insulin should be diluted with normal saline immediately prior to its intravenous injection, to give a solution containing 1 unit per mL (normally 10 units Velosulin diluted with saline in a 10 mL syringe). It should be given immediately after both baseline blood samples have been taken.
10. When adequate hypoglycaemia has been established (< 2.2 mmol/L or a 50% reduction in the baseline level), or if the child shows signs of hypoglycaemia (e.g. is sweaty and drowsy), a glucose drink should be given - see 2. above. If this is not tolerated, or if there are more severe symptoms of hypoglycaemia (impaired or loss of consciousness), intravenous glucose may be required - see 4. above.

11. Take further blood samples for glucose, cortisol and GH determinations at 20, 30, 60, and 90 minutes after the insulin injection. Check the glucose concentration in each sample as it is collected, using the ward meter, or more frequently if clinically indicated - see 3. above.
12. Give a sweet drink and a meal after the test and ensure that the meal has been eaten. Keep the child under observation for at least 1 hour after the meal has been consumed. Keep the intravenous catheter in position until lunch has been assimilated. Ensure that a blood glucose measured on the ward meter reads greater than 4 mmol/L before discharge. If there is any doubt about the child's wellbeing, keep him/her in overnight for observation.

Interpretation

If the plasma glucose falls to 2.2 mmol/L or less, or there is a 50% or more fall in blood glucose, the imposed stress should be sufficient for maximal stimulation of GH. Interpretative limits are as follows:

- A GH peak of 7.7 ug/L or above can be considered normal.
- A GH peak below 5 ug/L can be considered abnormal, this is diagnostic of GHD
- A GH peak below 3.3 ug/L is diagnostic of severe GHD. Patients may be candidates for ongoing treatment into adult life.
- A GH peak between 5 and 7.6 ug/L requires interpretation in context and one of the following 3 approaches can be taken:
  a) Reviewing the child's growth velocity in clinic over the period of 6 months to 1 year
  b) Performing a second GH provocation test
  c) Considering a trial of GH treatment for 1 year with rigorous re-assessment of their growth velocity and consideration given to stopping treatment if there is no improvement.

Hypoglycaemia of this magnitude should also cause an increase in the plasma cortisol to concentrations exceeding 400 nmol/L (all ages; based on 2017 audit).

Synacthen Screening Test

The synthetic polypeptide Synacthen (Tetracosactrin BP) has a structure identical to the N-terminal 24 amino acids of ACTH (Adrenocorticotrophic Hormone). It has a short duration of action and permits a rapid and convenient screening test for the assessment of adrenocortical function.

Measurement of adrenal steroids during the test may also be used to assess the steroid biosynthetic pathway. Plasma 17-hydroxyprogesterone (17-OHP) measurements may assist in the diagnosis of non-salt-losing congenital adrenal hyperplasia. If a defect in steroid biosynthesis is suspected, collect a basal urine for steroid profile for 24h prior to the synacthen test. Post synacthen urine steroid profile sampling is not usually necessary.

Procedure

For Synacthen tests carried out on children with asthma on high dose inhaled corticosteroids to assess adrenal function, refer to the Respiratory Medicine Clinical Protocol for this test.

1. Prednisolone and prednisone interfere with the measurement of cortisol. If the patient is taking either of these drugs they should be withheld for 24 hours (minimum 12 hours), or the patient switched to dexamethasone at least two days before the test. Any current steroid therapy should be noted on the Endocrine Function Test request form, including all inhaled, topical, nasal and/or oral steroids.
2. The patient need not be fasting. Insert an indwelling catheter at least 30 minutes before taking the baseline sample.
3. Collect blood for basal plasma cortisol (0.5 mL LiHep), (plus 17-OHP, androstenedione and DHAS if required: collect an extra 1.0mL Li Hep).

4. Give Synacthen (for children, 250 micrograms; for infants less than 7kg, 36 micrograms per kg body weight, rounded to the nearest 25 micrograms) by intravenous injection, and collect further blood samples for cortisol (and if indicated 17-OHP & androstenedione) 30 and 60 minutes later.

Interpretation

The peak cortisol response usually occurs at 60 minutes. Following recent review of Synacthen testing in Lothian, a single age-defined cut-off is now in use; an adequate peak cortisol response is >430 nmol/L (all ages).

Interpretation of plasma 17-OHP and androstenedione depends on age and clinical presentation. Both adults and children usually have baseline 17-hydroxyprogesterone concentrations less than 6 nmol/L and the increment in response to Synacthen should be less than 4 nmol/L. In late onset congenital adrenal hyperplasia, the baseline 17-hydroxyprogesterone may be either normal or high, but there is a grossly exaggerated response to Synacthen stimulation. Basal androstenedione concentrations are less than 2 nmol/L in children prior to adrenarche and little response is seen to Synacthen. After adrenarche, basal androstenedione concentrations are less than 9 nmol/L, and a moderate response to Synacthen may be observed.

Water Deprivation Test

During a period of restricted water intake in normal individuals, anti-diuretic hormone (vasopressin, ADH) is secreted by the posterior pituitary gland and facilitates renal tubular water re-absorption. This maintains plasma osmolality and concentrated urine is passed. The full test consists of a dehydration phase and a desmopressin phase. Only children who fail to concentrate their urine normally during the dehydration phase should proceed to the desmopressin phase, which involves administration of DDAVP (desmopressin, 1-desamino-8-D-arginine vasopressin) to determine whether the cause is of renal or pituitary origin. [Bayliss & Cheetham. Arch Dis Child 1998;79:84-89]

Procedure

Dehydration phase

1. Inform the laboratory before starting the test, so all specimens can be analysed promptly and the results telephoned to the ward without delay.
2. Fluids should ***not*** be restricted overnight before the test.
3. Start the test at 0800-0900h. Empty the patient's bladder. Measure the volume of urine and send to the laboratory promptly for osmolality determination. If this urine has an osmolality greater than 750 mmol/kg, water deprivation is unnecessary and the test can be terminated.
4. The patient may have a light breakfast but *no fluids*. Weigh the patient, and withhold fluids for the next 8 hours. Constant supervision is necessary to prevent surreptitious water drinking. Collect each urine specimen passed and measure the volume. Put an aliquot of each specimen into a separate, fully labelled container, and send to the laboratory for osmolality. Send each specimen to the laboratory promptly, because the test need not be continued once urine with an osmolality greater than 750 mmol/kg has been passed.
5. Plasma osmolality should also be measured at, for example, 2-hourly intervals and at the end of the 8 hour period of fluid deprivation.
6. Weigh the patient at frequent intervals during the test (e.g. hourly) and discontinue if the weight loss exceeds 5% of the body weight, or if a lesser weight loss is associated with clinical signs of dehydration.

Desmopressin phase

If an 8-hour period of fluid deprivation has *not* resulted in a urine osmolality greater than 750 mmol/kg, or if the test has been curtailed because of excessive weight loss or clinical signs of dehydration, proceed to the desmopressin phase of the test. Provided adequate supervision

can be guaranteed this can be done immediately as detailed below. If not the desmopressin phase should be carried out on a separate day.

7. Administer desmopressin (10 micrograms intranasally in infants, 20 micrograms intranasally in older children) at about 1600-1700h.
8. Allow the patient to eat and drink up to 1.5 times the volume of urine passed during the dehydration phase and beyond.
9. Collect blood and urine at about 2000-2100h. Measure urine volume and send both samples to laboratory promptly for measurement of osmolality (arrange with on-call BMS).
10. Fluids should *not* be restricted overnight.
11. Collect blood and urine at 0900h next morning. Measure urine volume and send both samples to laboratory for measurement of osmolality.

Interpretation

A normal response to the water deprivation test is defined as a urine osmolality >750 mmol/kg. Failure to concentrate urine during the dehydration phase, with an urine:plasma osmolality ratio <2.0 and often accompanied by an increase in plasma osmolality to greater than 295 mmol/kg is suggestive of diabetes insipidus of either cranial or renal origin (see table below). The desmopressin phase should discriminate between these two possible causes.

Urine osmolality (mmol/kg)

After fluid deprivation	After desmopressin	Diagnosis
>750	-	Normal or Primary (psychogenic) polydipsia
<300	>750	Cranial diabetes insipidus
<300	<300	Nephrogenic diabetes insipidus
300 - 750	<750	? Partial cranial diabetes insipidus ? Partial nephrogenic diabetes insipidus ? Primary (psychogenic) polydipsia

In long standing polyuria of any cause there may be renal resistance to the effects of vasopressin in both dehydration and desmopressin phases, resulting in an equivocal response.