Owner: Pediatric Department	Protocol Code: PR-PED-003
Title of The Protocol: Endocrine Dynamic Function Test Protocols For use in Neonates & Children	

Principles of Dynamic Testing

Basal or unstimulated hormone levels frequently do not provide enough diagnostic information in the investigation of endocrine disorders. A range of dynamic or provocative tests are available to assess the dynamic responses of hormonal and metabolic axes.

These tests may involve the following:

1. Stimulation of a hormonal axis by releasing hormones or other agents.
   e.g. Clonidine stimulation of growth hormone, Gonadotropin releasing hormone stimulation of LH and FSH …. etc.
2. Attempted suppression of a hormonal system e.g. cortisol suppression in Dexamethasone suppression test.
3. Physiological stimulation or challenge of a metabolic or hormonal system e.g. exercise stimulation of growth hormone, fasting study to assess glucose homeostasis, water deprivation to assess water regulation.

Safety considerations

Any dynamic or provocative test has potential for side effects or adverse reactions; although these are uncommon in experienced hands and if appropriate precautions are taken. Precautions, contraindications and adverse reactions are indicated in the protocols for each test and should be reviewed before each test is undertaken.

Important adverse reactions in various tests might include:

- Hypoglycemia.
- Dehydration.
- Minor reactions to provocative agents e.g. nausea, vomiting.
- Allergic or anaphylactic reaction to provocative agent.
- Cannula related complications including blood loss, infection…. etc.
- Hypotension.

To minimize potential adverse events the following should be considered:

- Tests should only be performed and supervised by personnel and centers experienced in their use in children, and this should be in specialized Pediatric endocrine centers.

- Staff must have detailed knowledge of the test protocol and provocative agents.
- Specialized nursing staffs familiar with these tests are essential if they are to be performed safely and give accurate results.
- Tests must be performed in an environment where full Pediatric emergency resuscitation facilities and experience are available.
- Deaths and serious morbidity have been reported from such testing in inexperienced hands, particularly with insulin stimulation tests (Shah, Stanhope, Matthew. Hazards of pharmacological tests of growth hormone secretion in childhood. BMJ 304: 173- 4, 1992.) and fasting studies.
- It may be necessary to adjust protocols for individuals or circumstances, and the same protocol cannot automatically be safely applied to all patients.
- Prior to the test, consideration should be given to any customization or precautions required for the individual patient (This should be discussed with the consultant concerned).
- A medical officer must always be readily available, and in certain tests (e.g. insulin stimulation test) and must be immediately available in the ward.

Blood sampling:

- Most tests require the insertion of one IV cannula through which provocative agents are administered and or periodic blood samples drawn.
- A large vein in the cubital fossa is the preferred insertion site.
- Occasionally separate infusion and sampling cannula are required or desirable.
- Butterfly needles are useful for single samples but are not recommended where multiple samples are to be taken.
- Arterial sampling via cannula or needle/syringe should only be used if there is no alternative.

BODY SURFACE AREA CALCULATION:

Body surface area can be calculated by the formula below, or by reference to the attached nomogram.

$$\text{BSA } m^2 \qquad \sqrt{\frac{weight\,(\text{kg}) \; x \; height\,(\text{cm})}{3600}}$$

Ref: Mosteller RD. Simplified calculation of body surface area. N Engl. J Med 1987; 317:1098.

Clonidine Stimulation Test "GH provocative test"

Indication:

Diagnostic test of growth hormone secretion. Clonidine might be used in combination with other agents for double pharmacological stimulation of GH.

Rationale:

Clonidine is a selective alpha-agonist with central and peripheral actions. Its usual uses are in hypertension and migraine prophylaxis. Central actions are predominantly via alpha 2 adrenergic stimulation and it is a potent stimulus to GH release via GHRH secretion. In this test clonidine is administered orally and the GH response in peripheral blood is measured.

Contraindications:

Sick sinus syndrome compromises intra-vascular volume.

Dose: 150 micrograms per m^2 BSA orally (calculate amount to the nearest half tablet).

Adverse reactions:

Drowsiness usually seen within 1-2 hours post ingestion. Fall in blood pressure is expected of 10 mmHg of diastolic pressure about one hour, may last several hours usually resolved by the end of the test although in some children might last longer, so parents need to be informed on side effects of Clonidine. Effect will be prolonged in renal failure.

Clonidine, in the dose given, can result in orthostatic hypotension. Venous access for saline administration must be available. The patients should be seated or lying down for the whole duration of the test.

Hypotension, if present can usually be corrected by simply having the patient lie down and by giving ***normal saline 10-20 ml/kg over 30-60 minutes as rapid infusion*** in addition to the maintenance fluid as well child is advised to have plenty of water to drink, if blood pressure, still not corrected, to repeat another bolus of normal saline 10-20 ml/kg over 30-60 minutes infusion.

However, if necessary, ***dopamine 5 ug/kg/min in 1/2 Normal Saline can be administered.***

Preparation:

Preferably morning test, with nil by mouth (excepting water) from midnight (food intake suppresses GH secretion). However, a minimum fasting time of only 2 – 4 hours is required, and short fasting times should be applied in infants and young children.

Accurate height and weight measurements, allowing surface area calculation.

As soon as the test has been started, maintenance requirement of intravenous fluid should be commenced of 0.9 saline in order to avoid dehydration from prolonged fasting **(No Dextrose should be infused)**.

Method:

1. IV cannula inserted; check baseline glucose level.
2. Baseline BP at time 0, then at 30-minute intervals.
3. Child recumbent and resting during the test; may drink water.
4. Dose given with water after 0 sample collected.
5. Blood sampling as below indicated.
6. Child fed after test, and only allowed home after BP stabilized to normal levels (not sooner than 30 min completion of test).
7. A mild to moderate drop in blood pressure is expected.

Sample	0 min	Clonidine	30 min	60 min	90 min	120 min
Plasma glucose	S		S	S	S	S
GH	S		S	S	S	S

Interpretation:

General principles are: A peak GH is usually at around 120 minutes. Peak GH response < 10 ng/ml suggests GH deficiency

Notes: Abnormal responses are also seen in hypothyroidism, obesity, celiac disease, and steroid and phenothiazine therapy. In case of two pharmacological GH stimulations, the second agent should be administered at 75 minutes from the start of the test and the whole test should last for 210 minutes.

References:

- Gil-Ad I, Topper E, Larow Z. Oral Clonidine as GH stimulation test. Lancet. Aug, 1979; 278279.
- Burns JF, D Angelo LJ. Complications of clonidine supplement test for Pheochromocytoma. NEJM 1982; 307:756-7.

Clonidine & Glucagon GH Stimulation Test

Indication:

Combined two pharmacological growth hormone stimulation tests for diagnosis of growth hormone deficiency. In KAUH, the two preferred agents to be used are "Clonidine & Glucagon", however, any combination of agents is accepted globally.

Rationale:

Clonidine is a selective alpha-agonist with central and peripheral actions. Its usual uses are in hypertension and migraine prophylaxis. Central actions are predominantly via α_2 adrenergic stimulation and it is a potent stimulus to HGH release via GHRH secretion. In this test Clonidine is administered orally and the GH response in the plasma is measured.

Glucagon stimulates release of GH and ACTH by its effects on α_2-receptors and stimulating insulin release. The Glucagon stimulation test has been advocated as a safer test than insulin stimulation in young children and infants especially in the low dose version. The sensitivity of the test may be enhanced by addition of β-blockers (false negatives reduced by 10-15 %). In this test, Glucagon is administered SC or IM, and the response of cortisol and GH in peripheral blood is measured.

Doses:

Clonidine: 150 micrograms / m^2 BSA orally (calculate amount to the nearest half tablet).

Glucagon dose: 15 microgram/kg IM injection to a maximum of 1 mg.

Adverse reactions:

Clonidine:

- Drowsiness usually seen within 1-2 hours post ingestion; fall in blood pressure is expected of 10 mmHg of diastolic pressure is expected about one hour, may last several hours usually resolved by the end of the test.
- Effect will be prolonged in renal failure.
- Troublesome adverse reactions are rare.
- Clonidine may result in orthostatic hypotension.
- Venous access for 0.9 normal saline administration must be available.
- The patients should be seated or lying down for the duration of the test.

- Hypotension, if present can usually be corrected by having the patient lie down and by giving ***0.9 saline 10-20 ml/kg over 30-60 minutes as rapid infusion*** in addition to the maintenance fluid as well child is advised to have plenty of water to drink, if blood pressure, still not corrected, to repeat another bolus of normal saline 10-20 ml/kg over 30-60 minutes infusion.

  However, if necessary, ***dopamine 5 ug/kg/min in 0.45 Saline can be administered.***

Glucagon:

- Nausea, vomiting & abdominal pain.
- It may cause reactive hypoglycemia, usually 30-40 minutes after glucagon injection.
- It is essential that the patient be given a meal at the conclusion of the test, and that the meal is not vomited, glucose level should be checked before the patient is discharged.

Preparation:

- Preferably morning test, with nil by mouth (excepting water) from midnight (food intake suppresses GH secretion). However, a minimum fasting time of only 2 – 4 hours is required, and short fasting times should be applied in infants and young children. Accurate height and weight calculate body surface area (BSA).
- **Start child on intravenous fluid of 0.9 saline at the start of test (only saline, NO Dextrose in the drip).**

Sample	0 min	**Clonidine**	30 min	60 min	**75 min**	90 min	120 min	150 min	180 min	210 min
Plasma glucose	S		S	S	**Glucagon**	S	S	S	S	S
GH	S		S	S	no sample	S	S	S	S	S

Interpretation:

- Peak GH is usually at seen around 120 minutes. Peak GH response < 10 ng /ml suggests GH deficiency. Abnormal responses are also seen in hypothyroidism, obesity, celiac disease, steroid and phenothiazine therapy.

References:

1. Gil-Ad I, Topper E, Laron Z. Oral Clonidine as GH stimulation test. Lancet. Aug, 1979; 278279.
2. Burns JF, D Angelo LJ. Complications of clonidine supplement test for Pheochromocytoma. NEJM 1982; 307:756-7

L-Dopa & Glucagon GH stimulation test

Indication:

Combined two pharmacological growth hormone stimulation tests for diagnosis of growth hormone deficiency. The two agents could be used are "Levodopa & Glucagon", however, any combination of agents is accepted globally.

Rationale:

L-DOPA: Precursor to the neurotransmitters dopamine, noradrenaline, and adrenaline collectively known as catecholamines. L-DOPA can be manufactured and in its trade names include Sinemet, Parcopa, Atamet, etc.).

Glucagon: stimulates release of GH and ACTH by its effects on α 2-receptors and stimulating insulin release. The Glucagon stimulation test has been advocated as a safer test than insulin stimulation in young children and infants especially in the low dose version. The sensitivity of the test may be enhanced by addition of b-blockers (false negatives reduced by 10-15 %). In this test, Glucagon is administered SC or IM, and the response of cortisol and GH in peripheral plasma is measured.

Doses:

L-dopa: 500 mg orally (children 10 mg/kg) (preferably fasting). Each tablet of Sinemet contains 250 mg of L-Dopa, so please adjust the dose to the nearest ½ tablet if small child.

Glucagon: 15 microgram/kg S/C or IM injection to a maximum of 1 mg.

Adverse reactions:

L-Dopa:

Hypotension, especially if the dosage is too high.

Nausea, vomiting, disorientation and confusion.

Glucagon:

Nausea, vomiting& abdominal pain.

Reactive hypoglycemia, usually 30-40 minutes after glucagon injection.

It is essential that the patient be given a meal at the conclusion of the test, and that the meal is not vomited. Glucose level should be checked before the patient is discharged.

Preparation:

- Preferably morning test, with nil by mouth (excepting water) from midnight (food intake suppresses GH secretion). However, a minimum fasting time of only 4 hours is required and short fasting times should be applied in infants and young children.
- Accurate height and weight calculate body surface area (BSA).
- Start child on intravenous maintainace fluid at the start of test ***(only 0.9 saline, NO Dextrose in the drip, unless there is hypoglycemia, i.e. glucose reading of less than 60mg/dl), then to shift again to normal saline after hypoglycemia resolved, and to continue the stimulation test till the end of the procedure.***
- Blood pressure measurements every 30 minutes.
- Child expected to have frequent vomiting as side effects of both L-Dopa and Glucagon, so please inform the parents and in repeated vomiting, you might give anti-vomiting medication.

Sample	0 min	L-Dopa	30 min	60 min	**75 min**	90 min	120 min	150 min	180 min	210 min
Plasma glucose	S		S	S	**give Glucagon**	S	S	S	S	S
GH	S		S	S	no sample	S	S	S	S	S

Interpretation:

- Peak GH is usually at seen at 120 minutes. Peak GH response < 10 ng /ml suggests GH deficiency.
- Abnormal responses are also seen in hypothyroidism, obesity, celiac disease, steroid and phenothiazine therapy.

Reference:

Clinical Guide to Laboratory Tests, 3rd ed. Teitz ed., W.B. Saunders, 1995.

Clonidine & Insulin growth hormone stimulation test

Indication:

Diagnostic test of growth hormone deficiency as double pharmacological stimulation of GH.

Rationale:

Clonidine is a selective alpha-agonist with central and peripheral actions. Central actions are predominantly via α_2 adrenergic stimulation and it is a potent stimulus to HGH release via GHRH secretion. Insulin-induced hypoglycemia induces GH secretion through effects on central alpha -adrenergic pathways.

Doses:

Clonidine: 150 micrograms per m^2 BSA orally (calculate amount to the nearest half talet).

Insulin: 0.1 unit/kg diluted in 5 ml normal saline and given by slow intravenous injection over 1 min.

Adverse reactions:

Clonidine:

Drowsiness usually seen within 1-2 hours post ingestion; fall in blood pressure is expected of 10 mmHg of diastolic pressure is expected about one hour, may last several hours usually resolved by the end of the test. Effect will be prolonged in renal failure.

Insulin:

Symptoms of hypoglycemia are expected including pallor, sweating, hunger, headache, tiredness, hypoglycemic seizures. Deaths have occurred, some associated with inappropriate (excessive) glucose resuscitation.

How to manage the side effects of the drugs?

Clonidine:

- Venous access for saline administration must be available.
- The patients should be seated or lying down for the duration of the test.
- Hypotension, if present can usually be corrected by simply having the patient lie down and by giving ***normal saline 10-20 ml/kg over 30-60 minutes as rapid infusion*** in addition to the maintenance fluid as well child is advised to have plenty of water to drink, if blood pressure, still not corrected, to repeat another bolus of normal saline 10-20 ml/kg over 30-60 minutes infusion.

However, if necessary ***dopamine 5 ug/kg/min in 1/2 Normal Saline*** ***can be administered.***

Insulin:

If blood glucose dropped <3 mmol/l, please give 2-4 ml /kg of Dextrose 10%, as a bolus slowly over 3-5 minutes and then change type of the maintenance fluid from 0.9% normal saline to D5% 0.45 normal saline. Repeat blood glucose in 10 minutes, if still low, give another bolus of 2-4 ml /kg of Dextrose 10%, as a bolus slowly over 3-5 minutes and consult your senior.

Preparation:

Preferably morning test, with nil by mouth (excepting water) from midnight (because food intake suppresses GH secretion). However, a minimum fasting time of only 2 – 4 hours is required for infants and young children. Accurate height & weight measurements, allowing surface area calculation.

As soon as the test started, maintenance requirement of intravenous fluid should be commenced of 0.9 saline **(No Dextrose should be infused)** in order to avoid dehydration from prolonged fasting.

Sample	0 min	**Clonidine**	30 min	60 min	**75 min**	90 min	120 min	150 min	180 min	210 min
Plasma glucose	S		S	S	**Insulin**	S	S	S	S	S
GH	S		S	S	no sample	S	S	S	S	S

Interpretation:

General principles are: Peak GH response < 10 ng /ml suggests GH deficiency.

References:

Gil-Ad I, Topper E, Laron Z. Oral Clonidine as GH stimulation test. Lancet. Aug, 1979; 278279.

Insulin Tolerance GH & ACTH Stimulation Test

Background:

"Insulin tolerance test" (ITT), is the gold standard test for assessing the integrity of the hypothalamic-pituitary-adrenal axis. Stress, in this case hypoglycemia, leads to the secretion of the hypothalamic hormones growth hormone releasing hormone (GHRH) and corticotrophin releasing hormone (CRH) which in turn stimulate the pituitary to produce GH and ACTH. ACTH production is assessed by the measurement of adrenal cortisol production. This test is dangerous as it relies on the induction of symptomatic hypoglycemia which must be treated immediately if the symptoms become severe.

Indication:

This test is not routinely used at KAUH, although it is considered the gold standard test to assess the integrity of the hypothalamic-pituitary-adrenal axis.

Precautions:

- This test is potentially dangerous and is not carried out routinely at KAUH.
- This test should not be carried out in a child with a history of epilepsy or cardiac arrhythmias.
- This test should not be carried out on patients with severe pan hypopituitarism or hypoadrenalism.
- This test should not be carried out in a patient with a glycogen storage disorder.
- A doctor (Intern) must be present throughout this test with the patient being closely monitored for symptoms of hypoglycemia which may require treatment.

Side Effects:

Sweating, palpitations, impaired or loss of consciousness & seizure.

Preparation:

- The patient must be fasted overnight (2-4 hours for infants), although water is allowed. Ensure that glucose (10% dextrose) and hydrocortisone are available for i.v. injection if necessary.
- A glucose drink must be available.

Protocol:

- Children might develop severe hypoglycemic after insulin administration.
- Check glucose levels (by glucometer) at the time of every sample and observe the child continuously for symptoms of severe hypoglycemia.
- Check that the child is responsive at the time of every sample. If they do not respond, then follow instructions for the emergency management of hypoglycemia.
- Start the test between 0800h and 0900h.
- Weigh the patient and insert an indwelling cannula and take a basal blood sample for glucose, growth hormone and cortisol.
- Wait 30 minutes before taking the baseline sample for glucose, growth hormone and cortisol as cannulation may cause GH to rise.
- The patient should be resting throughout the test.
- Check glucose level by glucometer.
  - If glucose < 3.5 mmol/l do not administer insulin.
  - If glucose level between 3.5 – 4.5 mmol/l, then administer half the dose of insulin.
  - If glucose level > 4.5 mmol/l, then continue with the test as indicated.
  - Give an IV dose of 0.1 units / kg body weight by subcutaneous route.
  - ***Insulin dose should be reduced to 0.05 units / kg in patients who might be unduly sensitive to insulin, such as patients with suspected hypopituitarism, severe malnutrition, or those with a baseline blood glucose between 3.5 and 4.5 mmol/l.***
  - ***If blood glucose dropped <3 mmol/l, please give 2-4 ml /kg of Dextrose 10%, as a bolus slowly over 3-5 minutes and then change type of the maintenance fluid from 0.9% normal saline to D5% 0.45 normal saline. Repeat blood glucose in 10 minutes, if still low, give another bolus of 2-4 ml /kg of Dextrose 10%, over 3-5 minutes and consult your senior.***
  - Take further blood samples for glucose, growth hormone & cortisol at 15, 30, 60- & 90-min post insulin administration.

Sample	0 min	Give insulin	30 min	60 min	90 min	120 min
Plasma glucose	S		S	S	S	S
GH	S		S	S	S	S
Cortisol	S		S	S	S	S

Interpretation:

- Interpretation is only possible if adequate hypoglycemia (plasma glucose < 2.2 mmol/l) has been achieved.
- If the laboratory plasma glucose falls to 2.2 mmol/l or less, the imposed stress should be enough to stimulate a plasma GH concentration exceeding 10 ng/ml.
- Hypoglycemia of this magnitude should also cause an increase in the plasma cortisol to concentrations exceeding 430 nmol/l.

References:

Managed clinical network of Scottish Pediatric Endocrine Group Dynamic function test handbook for Clinicians January 2012

Galloway P.J., McNeill E., Paterson W.F. & Donaldson M.D.C. (2002) Safety of the insulin tolerance test. *Arch Dis Child* 87: 354-356

Combined Anterior Pituitary Function test "Glucagon, Synacthen &GnRH"

Background:

When multiple pituitary hormone deficiencies are suspected, it is practical and economical to carry out as many combined tests as possible.

Indication:

Investigation of known/suspected multiple pituitary hormone deficiencies.

Precautions:

- The GnRH test cannot be performed if the child has been primed with sex steroid to stimulate GH response.
- The test should not be performed on a patient with pheochromocytoma or insulinoma as it may provoke an attack.
- The test should not be carried out following starvation of > 48 hours or in the presence of a glycogen storage diseases (the inability to mobilize glycogen may result in hypoglycemia).
- The test should not be carried out in patients with severe hypocortisolemia (defined as 08.00am level <100 nmol/l).
- Thyroid function must be normal as thyroxin deficiency may reduce the GH and cortisol response.
- Patient should have water all the time to avoid dehydration.

Side Effects:

- Glucagon commonly result in nausea, abdominal pain and vomiting.
- In children with suspected hypopituitarism, prolonged fasting may induce hypoglycemia.
- Blood glucose should be checked by glucometer in these patients whenever a sample is taken.
- Asthmatic patients should be carefully monitored.

Preparation:

Patients should have water only for 8 hours prior to the test.

Owner: Pediatric Department	Protocol Code: PR-PED-003
Title of The Protocol: Endocrine Dynamic Function Test Protocols For use in Neonates & Children	

Doses:

Glucagon:

Glucagon is administered IM using a dose of 15 µg/kg of body weight (maximum of 1 mg).

Gonadotropin Releasing Hormone:

Give GnRH IV or IM in a total dose of 100 micrograms. Children <1 year should be given a dose of 2.5 micrograms/kg.

Synacthen:

- IV / IM bolus.
- Children <1 month of age dose of 36 micrograms/kg.
- Children 1 - 12 months dose of 125 micrograms.
- Children > 1-year dose of 250 micrograms.
- **Alternatively, a dose of 250 micrograms/m^2 BSA may beused for all age groups.**

Sample	0 min	Glucagon /GnRH /Synacthen simultaneously	30 min	60 min	90 min	120 min
glucose	S		S	S	S	S
GH	S		S	S	S	S
ACTH	S		S	S		
Cortisol	S		S	S	S	S
LH/FSH	S		S	S	S	S
Estradiol/ Testosterone	S		------	------	------	------
Prolactin	S		------	------	------	------
TSH/ fT4	S		------	------	------	------

Interpretations:

Peak GH after glucagon stimulation is usually seen around 120 minutes. Peak GH response < 10 ng /ml suggests GH deficiency. Abnormal responses are also seen in hypothyroidism, obesity, celiac disease, steroid and phenothiazine therapy. A normal response for synacthen stimulation is an increase in blood cortisol to a level of ≥430 nmol/l at 30 minutes. In children with suspected hypogonadotropic hypogonadism, Basal LH usually <1 IU/l. LH peak post-GnRH <5 IU/l. FSH peak greater than LH peak.

References:

Brooks C., Clayton P. & Brown R. (2005) Brook's clinical pediatric endocrinology, 5th edition. Blackwell publishing, Oxford.

Dexamethasone Suppression Test

Indications:

Evaluation of suspected Cushing's syndrome, or in androgen excess states where adrenal tumor is suspected.

Three variations of this test are listed:

1. **Overnight standard (low dose) dexamethasone suppression test:** Used as a simple screening test for Cushing's syndrome.
2. **Overnight high-dose dexamethasone suppression test:** Used in distinguishing the cause of Cushing's syndrome i.e. in differentiating Cushing's disease (pituitary ACTH hyper secretion) from other causes of Cushing's syndrome - ectopic ACTH or adrenal tumors. Now an often used alternative to the traditional standard (long) dexamethasone suppression test - easier and more reliable.
3. **Standard (long) dexamethasone suppression test:** Less commonly used now, but may still have some role, especially in evaluating suppressibility in androgen excess states.

Rationale:

Dexamethasone is a synthetic glucocorticoid which is not measured in cortisol assay. Through negative feedback mechanisms, the administration of dexamethasone normally causes reduced ACTH secretion via effects on the hypothalamic-pituitary axis, and hence decreased cortisol secretion. Since adrenal androgen production is also partially under the control of ACTH, these also are normally suppressed by dexamethasone. In pathological states of autonomous hormone production, these feedback responses are lost or impaired. In general, low dose tests are used to establish the diagnosis of Cushing's syndrome regardless of its cause. High dose tests are used to distinguish Cushing's disease (pituitary ACTH hyper secretion) from ectopic ACTH and adrenal tumors.

Contraindications:

Intercurrent acute illness, systemic infection, Allergy to ACTH.

Overnight Low-Dose Dexamethasone Suppression Test

Formulation: Dexamethasone tabs 0.5 mg, 4 mg (scored)

Dose: 0.5-1 mg/m^2 or 10 mcg/kg.

Notes:

The dosage may be increased 50-100% in marked obesity. In severe depression, escape from suppression has been reported.

Preparation:

Must have 0800 Blood sampling (see below) on that morning often performed on an outpatient basis.

Method:

- 0800 Blood sample collected (see below).
- Oral dexamethasone 0.5 -1 mg given at 2300 - 2400 hr. (at night).
- 0800 Blood sampling next morning.

Precautions:

- False positive results may be obtained following the use of drugs that accelerate dexamethasone metabolism including phenobarbital, phenytoin, carbamazepine, rifampin, rifapentine, ethosuximide, diltiazem or cimetidine (if possible, these should be stopped a few weeks prior to the test).
- Drugs that increase cortisol binding globulin (CBG) may falsely elevate cortisol results including estrogens.
- Dexamethasone clearance maybe reduced in patients with liver and / or renal failure.
- Dexamethasone should be used cautiously in a child with diabetes mellitus with measurements of blood glucose during the period of the test.
- The child shouldn't be on exogenous glucocorticoids during the test e.g. steroid creams, inhalers and eye drops.

Adverse Effects:

There is no significant effect of short-term dexamethasone use.

Interpretation:

In normal subject's plasma concentrations fall to < 140 nmol/l, while in Cushing's syndrome they remain > 280 nmol/l. This is a screening test and is only of value if suppression occurs.

Failure to suppress may occur in:

Normal subjects due to stress, intercurrent illness, obesity, psychiatric disorder, estrogen treatment. Failure to suppress should prompt further evaluation as clinically indicated.

References:

Ismail AAA. Biochemical investigation in Endocrinology, Academic press 1981; 78-79.

Absolver RN. Handbook of Endocrinology Tests in Adults and children. Yearbook, medical publishers 1978; 31-33.

Nieman L.K., Beverly M.K.B., Fondling J.W., Newell-Price J., Savage M.O., Stewart P.M. and Montori V.M. (2008) The Diagnosis of Cushing's Syndrome: An endocrine society clinical practice guideline. *JCEM* **93**:1526-1540.

Dias R., Storr H.L., Perry L.A., Isidori A.M., Grossman A.B. & Savage M.O. (2006) The discriminatory value of the low-dose dexamethasone suppression test in the investigation of pediatric Cushing's syndrome. *Horm Res* **65**(3): 159 – 162.

Overnight High-Dose Dexamethasone Suppression Test (HDDST)

Background:

This test is used in patients who have Cushing's syndrome established by screening, but with requirement for the etiology to be further identified.

The test works on the basis that in most situations the corticotroph tumor cells in Cushing's disease retain some responsiveness to the negative feedback of glucocorticoids, whilst tumors ectopically secreting ACTH will not. However, the HDDST maybe abnormal in healthy people and normal in patients with Cushing's syndrome and therefore may not be helpful in establishing the diagnosis. Indeed, for adults the pre-test probability of ACTH-dependent Cushing's syndrome being secondary to pituitary dependent Cushing's disease is 85-90%. The HDDST correctly identifies 69% of adult patients as having Cushing's disease. Since the diagnostic accuracy of this test in identifying Cushing's disease is less than the pre-test probability of making this diagnosis, this test is now rarely used. As ectopic causes of Cushing's syndrome are extremely rare in children, there is a very limited evidence base concerning the use of this test, although one group advocate the use of the low dose dexamethasone suppression test as an adequate alternative (with suppression of >30% being suggestive of Cushing's disease).

Indication:

To differentiate pituitary-dependent and ectopic causes of Cushing's syndrome.

Formulation:

Dexamethasone tabs 4 mg (scored) (Dexamethasone, Fisons)

Dose: 8 mg / m^2 BSA orally at 2300 - 2400 hrs.

Preparation:

Must have had 0800 blood sampling (see below).

Often performed on an outpatient basis.

Sample	0800 Day 0	0800 Day 1 (after dexamethasone)
Cortisol	S	S
ACTH	only if specified	only if specified

Interpretation:

In normal subject's plasma concentrations fall to < 140 nmol/l. In Cushing's disease plasma cortisol levels are reduced to less than 50% of baseline in 95% of patients. In ectopic ACTH syndrome or adrenal tumors, suppression is less marked or absent.

Standard (Long) Dexamethasone Suppression Test

Rationale:

Patients with a normal axis totally suppress cortisol secretion when treated with 2 mg per day of dexamethasone. Patients with Cushing's syndrome do not suppress on that dose. Patients with pituitary dependent Cushing's disease suppress on high dose dexamethasone (8-16 mg/day) while carcinoma, adrenal adenoma or ectopic ACTH patients do not suppress at any dose.

Indication:

For the definitive diagnosis of Cushing's disease, and for the initial differential diagnosis of the syndrome.

Formulation:

Dexamethasone tabs 0.5 mg, 4 mg (scored) (Dexamethasone, Fisons)

Dose:

Dexamethasone is administered successively in a low dosage, then high dosage as follows:0.5 mg orally q6hrly on days 3 and 4 (all ages and sizes), 2 mg orally q6hrly on days 5 and 6.

Low dose = 20 µg/kg/dose, high dose = 80 µg/kg/dose).

Owner: Pediatric Department	Protocol Code: PR-PED-003
Title of The Protocol: Endocrine Dynamic Function Test Protocols For use in Neonates & Children	

Method:

- At least two days of basal measurements are needed.
- On the third, fourth, fifth, and sixth day give dexamethasone, 0.5 mg orally every 6 hours (08:00, 14:00, 20:00, 02:00) (Low dose suppression).
- On the seventh, eighth, ninth, and tenth day give dexamethasone, 2 mg orally every 6 hours (08:00, 14:00, 20:00, 02:00) (High dose suppression).
- If needed, the test can be continued four more days, giving dexamethasone 4 mg orally every 6 hours.
- Collect 24-hour urines for urinary free cortisol and creatinine each day of the protocol.
- Obtain 2 Blood samples for ACTH and cortisol every morning at 08^{30}, 09^{00} am.
- Obtain Blood samples for cortisol 19:00, 19:30, 20:00 every night.

Sample	Day 1		Day 2		Day 3	Day 4	Day 5	Day 6
	0800	2400	0800	2400	0800	0800	0800	0800
Cortisol	S	S	S	S	S	S	S	S
ACTH	S	S	S	S	S	S	S	S
DHEAS & Testosterone	S	-	S	-	S	S	S	S
24-hour UFC	S		S		S	S	S	S

Interpretation:

- Normal subjects will have suppressed cortisol production on low dose, whereas patients with Cushing's syndrome do not. At the high dose, 90 % of patients with
- Cushing's disease (ACTH dependent Cushing's syndrome) will suppress, whereas patients with adrenal adenoma, carcinoma or ectopic ACTH syndrome will not.
- Androgen levels are similarly interpreted.

Reference:

Nieman L.K., Beverly M.K.B., Fondling J.W., Newell-Price J., Savage M.O., Stewart P.M. and Montori V.M. (2008)

The Diagnosis of Cushing's Syndrome: An endocrine society of clinical practice guideline. *JCEM* **93**:1526-1540.

Fasting Study

Indication:

Suspected hypoglycemic disorders, or monitoring progress in a known hypoglycemic disorder.

Rationale:

The diagnosis of hypoglycemia and the elucidation of its cause require a monitored fasting study when clinical information and baseline studies are inconclusive. Fasting is performed under carefully controlled conditions to determine whether hypoglycemia occurs during the fasting period, and if so, to elucidate the cause by analysis of the relevant metabolites. Studies need to be individually planned according to the age of the patient and the suspected disorder. In patients with a known hypoglycemic disorder on therapy, periodic fasting studies are performed to guide further management decisions.

Contraindications:

Recent or Intercurrent illness.

Adverse reactions:

- Potentially a very hazardous test.
- Severe or refractory hypoglycemia.
- Hypoglycemic seizures.
- Cardiac arrhythmias (fatty acid oxidation disorders).

Preparation:

Requires very close supervision.

Admit patient, non-fasted, remain on current medications.

Method:

IV cannula inserted; must be a reliable intravenous line.

Medical officer determines the maximum fasting time and time of commencement of study.

As a guide to appropriate maximum fasting times:

Neonates, infants < 3m	4 to 8 hours (usually miss 1 feed only)
Infants 3 to 6 months	8 to 12 hours
6 months to 2 years	12 to 16 hours
2 to 10 years	16 to 20 hours
> 10 years	16 to 24 hours

Sample collection:

- Medical staff will advise which metabolites are to be monitored during the fast.
- Blood and urine are collected at baseline.
- The frequency of subsequent measurements is dependent on age and the likely duration of fast.
- In general, infants under 6 months should have hourly glucose and young children 2 hourly.
- In older children where early hypoglycemia is not anticipated, blood glucose is measured 4 hourly for 8-12 hours, then 1-2 hourly depending on progress.
- Blood glucose measurements must be rapidly available.
- Other metabolites are usually measured 2 hourlies, except where a short fast is anticipated where they may be measured hourly.
- Hydration must be maintained during the study and subjects are given free access to water.
- All urine passed is tested by Ketodiastix for ketones.
- Urine samples are kept and frozen for metabolic screen - pre-fast, all urines during the fast and first urine post-fast.

Termination of fast:

The fast is terminated when hypoglycemia occurs (plasma glucose ≤ 3mmol/l) or the previously determined maximum fast time is completed. Blood sample for all metabolites is collected at this time.

Sample	0 min	4 hr.	8 hr.	10 hr.	12 hr.	14 hr.	16 hr.	18 hr.	20 hr.	22 hr.	24 hr.
Glucose	S	1-2 hourly depending on progress. Younger children 2 hourly initially. Babies and young infants usually hourly and sometimes ½ hourly.									
Insulin	S	Collect all again at termination for all during hypoglycemia attack.									
β-hydroxy butyrate	S										
Free fatty acids	S										
Lactate and pyruvate*	S										
Cortisol	S	Collect again at termination									
Growth hormone	S	Collect again at termination									
Urine for metabolic screen	S	Unless otherwise specified, collected at 0 (pre-fast), then all urines during fast, and first post-fast urine.									
Urine Ketodiastix for ketones	S	All urines to be tested.									

Interpretation:

Some general principles are as follows:

- The physiological response to fasting is that as blood glucose falls, plasma FFA and ketones rise and there is progressive ketonuria. serum insulin becomes suppressed.
- In the presence of hypoglycemia, cortisol & GH should normally be elevated.
- Hypoglycemia with Ketotic response is also seen in hypopituitarism or glucocorticoid deficiency, and in the exaggerated physiological state termed "Ketotic hypoglycemia".
- In hyperinsulinemia states, serum insulin does not suppress appropriately, and hypoglycemia occurs in the absence of a significant rise in FFA, β-hydroxybutyrate or urinary ketones.
- In disorders of hepatic β-oxidation of fatty acids, hypoglycemia occurs with suppressed insulin, elevated FFA and minimal or absent ketone response.

- Note that in young children, infants and babies, the fasting study will be of shorter duration, and metabolites should be collected 2 hourly from commencement, or 1 hourly if only a short fast is anticipated. Specific additional measures may be requested by medical staff in certain clinical circumstances.

Reference:

Morris AA, Thekekara A, Wilks Z, Clayton PT, Leonard JV, Aynsley-Green A. Evaluation of fasts for investigating hypoglycemia or suspected metabolic disease. Arch Dis Child 1996. Aug;75(2):115-119 10.1136/adc.75.2.115.

Glucagon test for children with hyperinsulinism

Indication:

persistent hypoglycemic requiring high GIR (>8 mg/kg/min).

Formulation:

Glucagon - lyophilized powder for reconstitution, administered by intramuscular or intravenous injection.

Dose: 15 micrograms/kg (maximum 1 mg) intravenous/ intramuscular.

Adverse reactions:

- Nausea, vomiting, abdominal pain.
- Rebound reactive late hypoglycemia.
- Persisting hypoglycemia in glucagon non-responsive conditions, necessitating intravenous glucose administration.

Preparation:

- Medical officer should be present.
- Often performed as part of a fasting study at the time of hypoglycemia.
- IV sampling cannula.

Method:

- When blood glucose less than 50 mg, to give glucagon 1 mg either IM or S/C, then monitor blood glucose every 10 minutes for 40 minutes; **if there is no increase in blood glucose by 20 minutes, terminate test and rescue with IV dextrose.**

Sample	0 minutes	10 minutes	20 minutes	40 minutes
Glucose	S	S	S	S
Insulin	S	------	S	S
C-peptide	S	-------	S	S

Interpretation:

A positive response is a rise of more than 30 mg/dL and indicates that the hypoglycemia is due to increased insulin action.

Reference:

Palladino, AA., Stanley, CA. A specialized team approach to diagnosis and medical versus surgical treatment of infants with congenital hyperinsulinism. Semen Pediatr Surg. 20(1):32-7, 2011 Feb.

Glucagon Stimulation Test (for pituitary function)

Indication:

As a test of GH and ACTH secretion; may be useful in infants and young children < 5 years or other situations in which insulin stimulation is contraindicated.

Background:

Glucagon stimulates release of GH and ACTH by its effects on a- receptors and stimulating insulin release. The Glucagon stimulation test has been advocated as a safer test than insulin stimulation in young children and infants especially in the low dose version. The sensitivity of the test may be enhanced by addition of b-blockers (false negatives reduced by 10-15 %). In this test, Glucagon is administered SC or IM (with or without pre-administration of a b-blocker), and the response of cortisol and GH in peripheral Blood is measured.

Contraindications:

Recent or intercurrent illness.

Warning:

Glucagon may cause severe reactive hypoglycemia, usually 30-40 minutes after glucagon injection.

It is essential that the patient be given a meal at the end of the test, and that the meal is not vomited.

Blood glucose level should be checked before the patient is discharged.

Glucagon dose: 15 microgram/kg IM injection to a maximum of 1 mg.

Adverse reactions: Nausea, vomiting, abdominal pain, hypoglycemia.

Preparation:

- Nil by mouth 4-6 hours (fasting should not be longer than this in infants and young children).
- Accurate weight & height measurements.
- IV sampling cannula.

Method:

- Baseline bloods collected; blood glucose measured in ward.
- Glucagon administered by IM injection.
- Sampling as illustrated in below table.
- Child fed and must have normal blood glucose prior to discharge.
- Observe minimum of 2 hours after test.

	0 min	60 min	90 min	120 min	150 min	180 min
Glucose	S	S	S	S	S	S
GH	S	S	S	S	S	S
Cortisol	S	S	S	S	S	S
IGF-I	S	----	----	----	---	-----

Interpretation:

A peak GH is usually at around 120 minutes Peak GH response < 10 ng/ml suggests GH deficiency.

IGF-I Generation Test

Indications:

Growth hormone is administered to the patient where there is strong suspicion of GH insensitivity.

This is generally indicated by short stature with low IGF-1 levels and a normal or high response to GH provocation tests. Growth hormone should stimulate the generation of IGF-1 which is measured in basal and stimulated blood samples. Failure of IGF-1 generation is suggestive of GH insensitivity, with a sensitivity of 77 – 91% and a specificity <97% . This test is generally useful only in detecting more severe cases of growth hormone insensitivity.

Rationale:

Under normal circumstances, GH administration over several days is associated with significant rises in serum IGF-I and IGFBP-3. In conditions with growth hormone insensitivity, these responses are absent or attenuated, depending on the severity of the defect.

Contraindications:

Intercurrent illness.

Formulation:

Recombinant human growth hormone.

Dose: Recombinant human growth hormone 0.1 IU/kg body weight daily by subcutaneous injection for 4 consecutive days.

Adverse reactions:

Rarely, edema may occur with initiation of GH therapy due to sodium and water retention.

Preparation:

Nil by mouth from midnight on day 1 and 5 until samples collected.

Method:

On day 1, the morning baseline samples collected after nil by mouth overnight. GH administration begins that evening before bed (usually administered at home by the family) and continues for 4 days.

On day 5, final Blood samples collected in the morning after nil by mouth from midnight.

Sample	Day 1	Day 5

Insulin-like growth factor-I (IGF-I)	S	S

In-

Male Reference Range	
Age (Years)	IGF-I (µg/L)
< 1 Year	13 - 138
1	18 - 176
2	23 - 212
3	28 - 247
4	34 - 282
5	40 - 316
6	46 - 349
7	53 - 382
8	60 - 414
9	68 - 443
10	75 - 469
11	83 - 490
12	90 - 505
13	96 - 514
14	101 - 516
15	104 - 512
16	107 - 502
17	109 - 488
18 - 20	109 - 472

Female Reference range	
Age (Years)	IGF-I (µg/L)
< 1 Year	16 - 143
1	19 - 160
2	22 - 178
3	25 - 198
4	29 - 219
5	34 - 244
6	39 - 271
7	45 - 302
8	52 - 336
9	59 - 371
10	67 - 407
11	75 - 440
12	82 - 467
13	89 - 488
14	94 - 501
15	98 - 505
16	101 - 502
17	102 - 493
18 – 20	103 - 478

terpretation:

An incremental increase in IGF-1 of >15 µg/l above the baseline level excludes severe GH insensitivity.

Reference:

Coutant R., Dörr H.G., Gleeson H. & Argente J. (2012) Limitations of the IGF1 generation test in children with short stature. *Eur J of Endocrinology* 166: 351 – 357 .

IGF-1 normal ranges for age, gender & Tanner staging

Gender	Tanner Stage	IGF-I (µg/L)				
		2.5%	25%	50%	75%	97.5%
Male	I	81	133	160	188	255
	II	106	212	277	332	432
	III	245	341	407	449	511
	IV	223	365	439	492	578
	V	227	309	356	412	518
Female	I	86	153	188	235	323
	II	118	190	247	323	451
	III	258	336	383	431	529
	IV	224	340	378	438	586
	V	188	277	339	395	512

Gonadotrophin Releasing Hormone (GnRH, LHRH) Test

Indications:

Investigation of pubertal disorders whether, precocious puberty or delayed puberty, investigation of hypogonadotropic hypogonadism suspected pre-pubertally and in monitoring of children with precocious puberty treated with GnRH analogues.

Rationale:

GnRH (also called LHRH) from the hypothalamus stimulates luteinizing hormone (LH) and follicle stimulating hormone (FSH) release from the pituitary gland. Evaluation of this response is important in the evaluation of disorders of puberty.

Contraindications:

Pregnancy (relative contraindication)

Formulation:

Gonadorelin (HRF) 100 micrograms (plus 2 ml diluents); 500 micrograms (plus 2 ml diluents) A synthetic decapeptide identical to the naturally occurring hormone.

Dose:

Slow intravenous injection over 1 min.

2.5 micrograms/kg for children less than 1 year.

100 micrograms as single dose for children above 1 year.

Adverse reactions:

Significant adverse reactions have not been encountered. Occasionally nausea and abdominal pain.

Preparation:

Nothing, to be done at any time.

Method:

- Fasting is not necessary.
- If menstrual cycle is regular it is best to perform this test during the follicular phase.
- Immediately after taking the '0' Blood, inject GnRH 100 mg IV as bolus.
- Continue drawing samples as indicated below.

Sample	0	15	30	45	60	90	120

	min	min	min	Min	min	min	min
LH	S	S	S	S	S	S	S
FSH	S	S	S	S	S	S	S
Testosterone (M) Estradiol (F)	S	-	-	-	-	-	-

Interpretation:

The GnRH test should be interpreted in the clinical context (including pubertal staging, testicular volume/ovarian ultrasound) and with other biochemical markers of puberty such as serum estradiol or testosterone levels.

Prepubertal response:

Basal LH usually <1 IU/l.

LH peak post-GnRH <5 IU/l. FSH peak greater than LH peak.

Pubertal delay and pubertal failure:

In children with suspected hypogonadotropic hypogonadism, a complete lack of response

A measurable but low response has limited predictive value (may also occur in constitutional delay of puberty).

In primary gonadal failure, the basal LH & FSH are elevated and the response to GnRH is exaggerated. High basal FSH levels in the presence of low estradiol levels may suggest ovarian failure.

Premature thelarche:

There may be an FSH predominant response, with LH usually in the pre-pubertal range.

Precocious puberty:

- In GnRH-independent precocious puberty, spontaneous gonadotrophin secretion is suppressed by the autonomous sex steroid secretion: basal LH and FSH are low and the response to GnRH is flat.
- In GnRH-dependent precocious puberty basal LH and FSH levels are usually elevated and the response to GnRH is exaggerated.

- LH dominant rise is usually observed, with LH levels usually >7 IU/l and more commonly >10 IU/l in established puberty.

As monitoring during treatment of central precocious puberty:

Suppressed basal LH and FSH and flat response to GnRH indicate adequate.

References:

1. Resende E.A., Lara B.H., Reis J.D., Ferreira B.P., Pereira G.A. & Borges M.F. (2007) Assessment of basal and gonadotropin-releasing hormone-stimulated gonadotropins by immunochemiluminometric and immunofluorometric assays in normal children. JCEM 92:1424-9

2. Brito V.N., Batista M.C., Borges M.F., Latronico A.C., Kohek M.B., Thirone A.C., Jorge B.H., Arnhold I.J. & Mendonca B.B. (1999) Diagnostic value of fluorometric assays in the evaluation of precocious puberty. JCEM 84: 3539-44

3. Trueman J.A., Tillmann V., Cusick C.F., Foster P., Patel L., Hall C.M., Price D.A. & Clayton P.E. (2002) Suppression of puberty with long-acting goserelin (Zoladex-LA): effect on gonadotrophin response to GnRH in the first treatment cycle. Clin Endocrinol (Oxf). 57: 223-30.

HCG Simulation Test

Indications:

A test to determine the Leydig cell responsiveness of the testes.

Rationale:

HCG induces an increase in testosterone biosynthesis and secretion by Leydig cells which can be measured within several days of administration. It is most commonly used in suspected primary Hypogonadism or identifying the presence or absence of testicular tissue in cryptorchidism. While HCG stimulates ovarian estrogen and progesterone secretion, it is not employed as a diagnostic test in females.

Contraindication:

Known or suspected androgen sensitive tumors (usually mammary carcinoma or prostatic carcinoma in the male)

Formulation:

- Human chorionic gonadotropin - lyophilized powder for reconstitution, administered by intramuscular injection.
- Pregnyl (Organon) - 500 IU, 1500 IU, 5000 IU.
- Profasi (Serono) - 500 IU, 1000 IU, 2000 IU, 5000 IU.

Dose:

- 500 IU if weight < 5kg.
- 1000 IU if weight 5 - 10kg.
- 1500 IU if weight 10 - 15kg.
- 3000 IU if weight above 15kg.

Adverse reactions:

Skin rashes, local reaction (both rare).

Other side effects related to prolonged and high dose administration only.

Preparation:

Nil could be done at any time.

Sample	pre-hCG 0 hours	post-hCG 72-96 hrs
Testosterone & Di-hydrotestosterone	S	S
LH	S	S*
FSH	S	-

Interpretation:

- Normal testosterone response if stimulated testosterone level more than 3-fold baseline, or if base-line level < 1 nmol/l then peak > 4 nmol/l.
- A lesser response suggests Leydig cell failure or absence.
- Normal T/DHT ratio 10 (mean) (range 2-20).
- Poor DHT response and elevated post- hCG T/DHT ratio suggests 5-α- Reductase deficiency.
- In patients with DSD, a rise in testosterone in response to hCG stimulation, indicates the presence of testicular tissue and a radiological approach to find out this tissue should be investigated.
- In males with cryptorchidism, a rise in testosterone post hCG stimulation confirms the presence of testes.

References:

1. Maimoun L., Philibert P., Cammas B., Audran F.,et al. Phenotypical, Biological and molecular heterogeneity of 5α-Reductase deficiency: An extensive international experience of 55 patients. *JCEM* **96**: 296 - 307
2. Segal T.Y., Mehta A., Anazodo A., Hindmarsh P.C. & Dattani M.T. (2009) Role of gonadotropin-releasing hormone and human chorionic gonadotropin stimulation tests in differentiating patients with hypogonadotropic hypogonadism from those with constitutional delay of growth and puberty. *JCEM* **94**(3): 780 – 785.

Intravenous Glucose Tolerance Test

Indication:

Study of the dynamics of insulin secretion, usually in suspected pre- diabetes or glucose intolerance. A test of longer duration may be used to study insulin sensitivity.

Background:

This test provides a detailed assessment of the dynamics of insulin secretion and glucose disappearance. A known bolus of glucose is injected, and samples collected at specified intervals for measurement of plasma glucose and insulin. Several indices can be calculated from the data, but most commonly first phase insulin release is employed. The test described here is the consensus reached by the ICARUS (Islet cell Antibody Register User's Study) working group (reference: Bingley et al, Diabetes Care, 1992, 15:1313-16). A more prolonged test with sampling to 40 or 60 minutes can be used to measure insulin sensitivity, but this is not a routine clinical test.

Contraindication:

Hyperglycemia, overt diabetes.

Formulation:

50 % Glucose; ampoules.

Dose:

0.5 g/kg body weight to a maximum of 35g to be administered as a 25 % solution by dilution with equal volume of sterile water.

Adverse reactions:

- A flushing sensation may occur during glucose infusion.
- Adverse reactions are not expected.

Preparation:

- Unrestricted diet (containing at least 150 g carbohydrate per day) for at least 3 days prior.
- Normal physical activity, no significant intercurrent illness.
- Test performed in the morning after an overnight fast of 10 - 16 hours.
- Accurate weight measurement.

Method:

- IV cannula(e) inserted and patient rests 30 mins.
- Dose calculated and glucose diluted to 25 % with sterile water.
- Pre-glucose samples collected as below.
- Glucose injected over 3 minutes ± 15 seconds, using timer (calculate amount to be injected every 30 seconds as a guide for injection rate).
- Time 0 is immediately after the glucose injection.
- Then flush cannula with 20 ml normal saline over 15- 20 seconds (important to flush all glucose through before sampling begins especially if using one cannula) and prepare for sampling at 1 minute by commencing to withdraw void volume 10 seconds before the 1-minute mark.
- IV glucose to be injected by medical officer or accredited nurse.
- Child recumbent and resting during the test.
- Water is permitted.
- Collect samples at accurately timed intervals as below.
- Note that samples are closely spaced, and timing is critical.

Sample	-5 min	0 min = Immediately after glucose Injection. Mark time and begin saline flush	1 Min	3 min	5 min	10 Min
Glucose	2. S	No sample	S	S	S	S
Insulin	S	No sample	S	S	S	S

Interpretation:

The sum of the insulin levels at 1 and 3 minutes is termed the first-phase insulin secretion. The first percentile for first phase insulin secretion is 48 mU/l (360 pmol/l) (reference Vardi et al, Diabetologia, 1991, 34:93-102) and values below this are very likely to be abnormal, but must be considered in association with other available clinical and laboratory information (e.g. family history, ICA titer).

Since there is a coefficient of variation of 36 % within subjects, changes within the normal range (> 5th percentile) cannot be interpreted as pathological.

Oral Glucose Tolerance Test (oGTT)

Background:

In normal individuals, pancreatic insulin secretion maintains blood glucose within a tight concentration range following an oral glucose load. Failure of insulin secretion, or resistance to insulin action, will result in an elevation in blood glucose. The Glucose Tolerance Test is usually used to exclude/confirm a diagnosis of Glucose intolerance or type 2 Diabetes Mellitus. The test is unnecessary if a child has characteristic symptoms of diabetes (e.g. weight loss, thirst, and polyuria) and either a random venous plasma laboratory glucose concentration of ≥ 11.1 mmol/l, or a fasting concentration of ≥ 7.0 mmol/l.

Indication:

The oral glucose tolerance test is used to clarify borderline elevation in fasting plasma glucose. The oGTT is not indicated when a patient has an unequivocally elevated fasting or random plasma glucose. An oGTT only needs to be performed in a child with an equivocal result for the diagnosis of diabetes.

Precautions:

- This test is only necessary if fasting glucose measurements are equivocal i.e. 5.6 - <7.0 mmol/l.
- This test should not be performed in patients who fulfil the criteria for diabetes mellitus: Two diagnostic glucose results on separate occasions (either fasting plasma glucose ≥ 7.0 mmol/l or random plasma glucose of ≥ 11.1 mmol/l), or one diagnostic glucose result and clinical symptoms of diabetes e.g. polydipsia, polyuria, ketonuria and rapid weight loss.
- Do not perform glucose tolerance tests on patients with uncontrolled thyroid dysfunction or patients who are under physical stress e.g. post-surgery, trauma or infection or extreme psychological stress as these may give misleading results due to alternated insulin sensitivity in these circumstances.
- This test should also not be performed on patients with hypokalemic periodic paralysis.

Adverse effects:

Some patients feel nauseated and may have vasovagal symptoms during this test.

Preparation:

- Before subjecting a patient to an OGTT ensure that there has been an appropriate diagnostic work-up (see WHO guidelines).
- Ensure that the child has had an adequate diet (minimum of 150 g/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.
- Physical exercise is not allowed in morning prior to and/or during the test. Test should be performed in the morning.

Protocol:

- Ensure the patient's fasting blood glucose concentration, checked with a capillary blood sample obtained by finger prick testing with a glucometer, is ≤ 7 mmol/l before proceeding with the test. If the result is higher, take a venous blood sample and send it to the lab to confirm the glucometer result.
- Prepare the glucose load using glucose: Dose 1.75 g/kg body weight (maximum dose 75g diluted in 200 mL water).
- The child should drink the glucose load over a period of no more than 5 min. Note the time the glucose load is given on the request form.

Sample	0 min	30 min	60 min	90 min	120 min	150 min	180 min
Glucose	S	S	S	S	S	S	S
Insulin	S	--	--	--	--	S	S

Interpretation:

The flow chart on the following page indicates the diagnostic criteria for Diabetes mellitus.

Venous plasma:

- A fasting glucose level of > 7 mmol/l or a level of > 11.1 mmol/l, 120 min post-glucose load confirms a diagnosis of diabetes mellitus.
- Levels between 7.8 – 11.0 mmol/l, 120 min post glucose load indicate impaired glucose tolerance.

Values for diagnosing diabetes using different sample types are indicated in the table below:

	Glucose Concentration (mmol/L)		Glucose Concentration (mmol/L)	
	Whole blood		Plasma	
	Venous	Capillary	Venous	Capillary
Diabetes Mellitus				
Fasting	≥6.1	≥6.1	≥7.0	≥7.1
120 min post-glucose	≥10.0	≥11.1	≥11.1	≥12.2
Impaired glucose tolerance				
120 min post-glucose	≥6.7 and <10.0	≥7.8 and <11.1	≥7.8 and <11.1	≥8.9 and <12.2
Impaired fasting glycaemia				
Fasting	≥5.6 and <6.1	≥5.6 and <6.1	≥6.1 and <7.0	≥6.1 and <7.0

References:

1. East Kent Hospitals University NHS Foundation Trust Clinical Biochemistry: OGTT – Protocol for pediatrics.

2. Colley C.M. & Larner J.R. (1990). The use of Fortical in glucose tolerance tests. Ann Clin Biochem 27: 496 – 498.

3. WHO/IDF report (2006) Definition and diagnosis of diabetes mellitus and intermediate hyperglycemia.

4. Brooks C., Clayton P. & Brown R. (2005) Brook's clinical pediatric endocrinology, 5th edition. Blackwell publishing, Oxford.

Metyrapone Test

Background:

Metyrapone can be used in the diagnosis of adrenal insufficiency. Metyrapone test may aid in verifying

the cause of Cushing's syndrome. Most patients with pituitary dysfunction and/or pituitary microadenoma will increase ACTH secretion in response to metyrapone, while most ectopic ACTH-producing tumors will not. Pituitary macroadenomas do not always respond to metyrapone.

Metyrapone is used for the medical control of hypercortisolism in Cushing's syndrome (ACTH dependent or independent). The aim for medical treatment is to achieve pre-operative control of hypercortisolism, or for control of residual disease persisting post-operatively (TSS, adrenalectomy). It is not for long term definitive treatment/cure, only as an adjunct (surgery is the aim for cure in most causes of Cushing's syndrome). Metyrapone hence acts by inhibiting adrenal steroidogenesis. One side effect is hirsutism (in women) because of the excess androgen precursors created. The other commonly used agent for medical treatment of Cushing's is ketoconazole (an anti-fungal agent). This does not exhibit the side effect of hirsutism.

Rationale:

Metyrapone blocks cortisol biosynthesis by acting as a reversible inhibitor of 11β-hydroxylase. This stimulates adrenocorticotropic hormone (ACTH) secretion, which in turn increases plasma 11-deoxycortisol levels.

Indication:

For evaluating pituitary ACTH reserve, particularly when insulin hypoglycemia is contraindicated.

Contraindications:

In suspected Addison's disease, Metyrapone may precipitate Addisonian crisis, although this is rare with the single dose protocol.

Dose:

Metyrapone 30 mg/kg as single oral dose.

Warnings:

Metyrapone may cause gastric upset and should be given with food or milk.

Method:

Metyrapone 30 mg/kg, maximum dose 3,000 mg, is administered at midnight usually with a snack. The

plasma cortisol and 11-deoxycortisol are measured the next morning between 8:00 and 9:00 am.

Interpretation:

A plasma cortisol less than 220 nmol/l indicates adequate inhibition of 11β-hydroxylase.

In patients with intact Hypothalamic-pituitary-adrenal axis, CRH and ACTH levels rise as a response to the falling cortisol levels. This results in an increase of the steroid precursors in the pathway. Therefore, if 11-deoxycortisol levels do not rise and remain less than 7 µg/dl (202 nmol/l) and adrenocorticotropic hormone (ACTH) rises, then it is highly suggestive of adrenal insufficiency. If neither 11-deoxycortisol nor ACTH rise, it is highly suggestive of an impaired hypothalamic–pituitary–adrenal axis at either the pituitary or hypothalamus.

References:

1. Young EA, Ribeiro SC, Ye W (June 2007). "Sex Differences in ACTH Pulsatility following Metyrapone Blockade in Patients with Major Depression". Psychoneuroendocrinology. **32** (5):503–7. doi:10.1016/j.psyneuen.2007.03.003.
2. Marin, Marie-Frances; A. Hupbach; F. S. Maheu; K. Nader; S. J. Lupien (2011). "Metyrapone Administration Reduces the Strength of an Emotional Memory Trace in a Long-Lasting Manner". Journal of Clinical Endocrinology & Metabolism. early release abstract (8): E1221–E1227. *doi*:*10.1210/jc.2011-0226*. *PMID 215931*.

Sex Steroid Priming in Growth Hormone Stimulation Tests

Rationale:

Sex steroid priming prior to GH stimulation is recommended in some centers, especially in subjects with delayed puberty, although is uncommonly used at our Institute. The rationale for priming is that a proportion of prepubertal children with slow growth (especially those with delayed puberty) may have subnormal GH levels during stimulation, but when they are re- tested later or after onset of puberty, normal levels are found. This may avoid some false diagnoses of GH deficiency and decrease the number of additional tests required but does not really aid in determining which subjects are GH-insufficient. If used, should generally be reserved for patents with delayed puberty.

Contraindications:

Age < 10 years (relative contraindication)

Precocious puberty

Formulations:

Males:

Testosterone depot preparation 100 mg IMI given 2-8 days prior to test.

or testosterone undecanoate (Andriol) 80 mg daily for 5 days prior to test.

Females:

Ethinyl Estradiol 50 - 100 µg daily for 3 days prior to test.

Adverse reactions:

Significant side-effects are not anticipated, except possible minor manifestations of temporarily increased sex steroid levels.

Short ACTH (Synacthen) Stimulation Test

(for suspected Adrenal insufficiency)

Indications:

Owner: Pediatric Department	Protocol Code: PR-PED-003
Title of The Protocol: Endocrine Dynamic Function Test Protocols For use in Neonates & Children	

To assess the response of the adrenal cortex to stimulation in suspected adrenocortical insufficiency (primary, secondary or tertiary) or in the diagnosis of congenital adrenal hyperplasia.

Rationale:

ACTH is the primary regulator of glucocorticoid production and plays some role in adrenal androgen production. Synacthen is a synthetic form of ACTH, is used to assess the stimulated cortisol response of the adrenal cortex and is valuable in diagnosing suspected primary adrenal insufficiency. The test is also useful in suspected secondary or tertiary adrenal insufficiency since chronic CRH/ACTH deficiency or dysregulation results in temporary quiescence of the adrenal cortex and inability to respond acutely. The test is not reliable in assessing secondary or tertiary insufficiency within 4 weeks of surgery to the hypothalamic-pituitary region or a major alteration in any glucocorticoid therapy. An 8 - 9 am plasma ACTH and cortisol can be informative in these situations.

In congenital adrenal hyperplasia, Synacthen test is useful in diagnosing milder or rare enzyme blocks by examining ratios of various adrenal steroids to their precursor compounds. The commonest ratio examined is that of 17-hydroxyprogesterone / cortisol in suspected non-classical or simple virializing CAH or the heterozygote state.

Contraindications:

Known hypersensitivity to ACTH.

Dose: Give Synacthen as an i.v. bolus

- For children <1 month use a dose of 36 micrograms/kg
- For children 1 - 12 months use a dose of 125 micrograms
- For children >1 year use a dose of 250 micrograms
- ***Alternatively, a dose of 250 micrograms/m^2 BSA may be used.***

Preparation:

- The test should preferably be performed in the morning between 0800 and 0900 hrs.
- The patient does not need to be fasted.
- All glucocorticoid therapy (other than dexamethasone or betamethasone) interferes with the assay of cortisol.
- If the patient is on prednisolone therapy, this must be discontinued for 24 hours prior to the test.
- If the patient is on a supra-physiological dose of hydrocortisone, this should be reduced to a physiological level (6 micrograms/m2 /day) prior to the test.

- Omit the dose the night before and on the morning of the test.
- If the pediatric endocrine consultant is very anxious about the degree of adrenal insufficiency, then omit only the morning hydrocortisone dose. However, the patient should take their usual dose of corticosteroid as soon as the test is completed.
- Take 1 ml of 250 µg/ml Tetracosactrin and dilute under sterile conditions with 49 ml of normal saline to make a concentration of 5 µg/ml.
- Take 1 ml of 5 µg/ml solution and 19 ml normal saline to make a 0.25 µg/ml solution. Do not store solution for later use.
- To calculate dose in ml of 0.25 µg/ml solution = (Patient BSA/1.73) x 2

Adverse reactions:

- Severe allergic reactions to Synacthen have been described, particularly in children with a history of allergic disorders, but are very rare. In children with prior known Synacthen sensitivity, a repeat
- Synacthen test is not advisable. In such cases, morning basal ACTH and cortisol levels can alternatively test for adrenal function.
- Nil at Any time of day.

Sample	0 min	30 min	60 min
Cortisol	S	S	S
ACTH	S*	-	-

Interpretation:

- A normal response is an increase in blood cortisol to a level of ≥430 nmol/l at 30 minutes.
- An impaired response does not distinguish between adrenal and pituitary failure, as the adrenal glands may be atrophied secondary to ACTH deficiency.

- The sensitivity of the Synacthen test is higher in primary adrenal insufficiency compared with secondary adrenal insufficiency. Sensitivity is particularly low in recent-onset ACTH deficiency (within 4 – 6 weeks of an insult to the pituitary).
- Cortisol results may be misleadingly low in the presence of low cortisol binding globulin (for example in severe illness, in conjunction with low albumin).
- In patients on long-term glucocorticoids it is difficult to differentiate underlying adrenocortical disorders from the adrenal-suppressive effects of the treatment.

References:

- Agha A., Tomlinson J.W., Clark P.M., Holder G. & Stewart P.M. (2006) the long-term predictive accuracy of the short synacthen (corticotropin) stimulation test for assessment of the hypothalamic-pituitary-adrenal axis. *JCEM*. 91: 43-7
- Klose M., Lange M., Rasmussen A.K., Skakkebaek N.E., Hilsted L., Haug E., Andersen M. & Feldt-Rasmussen U. (2007) Factors influencing the adrenocorticotropin test: role of contemporary cortisol assays, body composition, and oral contraceptive agents. *JCEM*. 92: 1326-33.
- Dorin R.I., Qualls C.R. & Crapo L.M. (2003) Diagnosis of adrenal insufficiency. *Ann Intern Med.* 139: 194-204.

Standard Dose Synacthen Test for Congenital Adrenal Hyperplasia (CAH)

Background:

Adrenal glucocorticoid secretion is controlled by adrenocorticotrophic hormone (ACTH) released by the

anterior pituitary. This test evaluates secretion of cortisol and 17-hydroxyprogesterone (17-OHP) by the adrenal cortex following stimulation with Synacthen. In patients with congenital adrenal hyperplasia (CAH; a group of inherited disorders caused by enzyme defects in the steroid synthetic pathway), cortisol may, or may not, be adequately secreted. However, there is excessive secretion of the precursor steroids proximal to the defective enzyme. The commonest cause of CAH is due to 21-hydroxylase deficiency and in these subjects increased secretion of 17-hydroxyprogesterone (17-OHP) occurs.

Indication:

Diagnosis of non-classical form of CAH due to 21-hydroxylase deficiency in children.

Precautions:

The Synacthen test gives unreliable results if performed within 4 weeks of pituitary surgery.

Adverse Effects:

Severe allergic reactions to Synacthen have been described, particularly in children with a history of allergic disorders, but are very rare. In children with prior known Synacthen sensitivity, a repeat synacthen test is not advisable. In such cases, morning basal ACTH and cortisol levels can alternatively test for adrenal function.

Preparation:

The test should preferably be performed in the morning between 0800 and 0900 hrs. The patient does not need to be fasted.

All glucocorticoid therapy (other than dexamethasone or betamethasone) interferes with the assay of cortisol. If the patient is on prednisolone therapy, this must be discontinued for 24 hours prior to the test. If the patient is on a supra-physiological dose of hydrocortisone, this should be reduced to a physiological level (6 micrograms/m2/day) prior to the test. Omit the dose the night before and on the morning of the test. If the pediatric endocrine consultant is very anxious about the degree of adrenal insufficiency, then omit only the morning hydrocortisone dose.

However, the patient should take their usual dose of corticosteroid as soon as the test is completed.

Doses:

- For children <1 month use a dose of 36 micrograms/kg.
- For children 1 - 12 months use a dose of 125 micrograms.
- For children >1 year use a dose of 250 micrograms.

- ***Alternatively, a dose of 250 micrograms/m^2 BSA may be used.***

Sample	0 min	30 min	60 min
Cortisol	S	S	S
17-OHProgesterone	S*	S*	S*
ACTH	S*	No	No
Androgens (DHEAS, Androstendione &, Testosterone)	only if indicated		

Interpretation:

- Unaffected children usually have a basal 17-OHP of < 6 nmol/l.
- A minority of patients with non-classical CAH have a normal basal 17-OHP, even on early morning samples.
- A normal response to Synacthen is a stimulated 17-OHP of <30 nmol/l at 60 minutes.
- A stimulated 17-OHP (60 minutes post-Synacthen) of 30 - 50 nmol/l is suggestive of CAH but some heterozygotes have levels within this range. Genotyping of the 21-hydroxylase gene may help reach a diagnosis.
- A stimulated 17-OHP of ≥50 nmol/l is consistent with a diagnosis of CAH.
- Milder elevations of 17-OHP may be found in rarer forms of CAH: 11-β-hydroxylase deficiency & 3-β-hydroxysteroid dehydrogenase deficiency.
- An increment of <10 nmol/l in normal individuals compared to >20 nmol/l in CAH has been reported.

- A normal cortisol response is an increase in plasma/blood cortisol to a level of ≥430 nmol/l at 30 minutes.

References:

1. Wilson R.C., Mercado A.B., Cheng K.C. & New M.I. (1995) Steroid 21-hydroxylase deficiency: genotype may not predict phenotype. *JCEM*. 80: 2322-9.

2. New M.I., Lorenzen F., Lerner A.J., Kohn B., Oberfield S.E., Pollack M.S., Dupont B.O., Stoner E., Levy D.J., Pang S. & Levine L.S. (1983) Genotyping steroid 21-hyroxylase deficiency: hormonal reference data. *JCEM.* 57: 320-326.
3. Bachega T.A., Billerbeck A.E., Marcondes J.A., Madureira G., Arnhold I.J. & Mendonca B.B. (2000) Influence of different genotypes on 17-hydroxyprogesterone levels in patients with non-classical congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *Clin Endocrinol (Oxf).* 52: 601-7.
4. Wallace A.M. (1995) Analytical support for the detection and treatment of congenital adrenal hyperplasia. *Ann Clin Biochem* 32: 9-27.

Water Deprivation Test

Indications:

Investigation of suspected diabetes insipidus

Rational:

Under normal circumstances, water deprivation is associated with declining urine volumes, increasing urine osmolality and maintenance of normal Blood osmolality. Such effects are mediated by increased ADH (vasopressin) secretion by the posterior pituitary and its action on the collecting ducts of the kidney. Water deprivation is most commonly used in patients presenting with polyuria and polydipsia to assist in distinguishing central diabetes insipidus, nephrogenic diabetes insipidus and psychogenic (habitual) water drinking. A test dose of Desmopressin may be given at the end of the test if needed to distinguish between central and nephrogenic DI.

Contraindications:

Existing dehydration or electrolyte abnormality.

Intercurrent illness.

Formulation:

Desmopressin (Minirin intranasal) 100 micrograms / ml.

Desmopressin injection 4 micrograms / ml (1 ml ampoules).

Adverse reactions:

Excessive water deprivation may cause significant dehydration and electrolyte disturbance, especially hypernatremia.

Desmopressin administration at the end of a test needs careful supervision to avoid over hydration and electrolyte disturbance.

Preparation:

Biochemistry laboratory notified in advance for multiple samples requiring rapid analysis.

Accurate weight (note clothing and other items included and calculation of 5% dehydrated weight (an indication to cease test)

Method:

An individual decision is made by the consultant on commencement time, considering age and anticipated rate of dehydration based on the clinical history. Where rapid dehydration may be anticipated or in young children, the test is commenced at 8 am. Where less rapid dehydration is expected (e.g. child normally sleeps through the night without drinking) the test may commence in the evening or sometime during the

night.

IV cannula inserted

Baseline weight, urine sample and Blood sample collected (see below).

No food or drink is allowed during the test, and the child must be observed to ensure that surreptitious water intake is prevented.

Samples collected and patient weighed at intervals as indicated. Thirst sensation and behavior during the test should be recorded.

Duration of water deprivation is seldom longer than 12-16 hours in children or adolescents and 6-8 hours in young infants.

In any case, the test is terminated if there is either:

- 5 % dehydration (5% weight loss), or Blood osmolality > 300 mosmol/l
- Children with normal responses to water deprivation (see below) are given drink at the end of the study and are allowed home after fluid and food has been taken.
- In those with inadequate urinary concentration, Desmopressin may be administered at the end of the test, however this is not routine. The primary aim of this is to distinguish central from Nephrogenic DI. It is recommended that where feasible,
- Test doses of Desmopressin are given during working hours. The dosage needs to be determined individually for the child by the supervising consultant.

Suggested doses:

- Neonates and infant: 1 - 2.5µg Desmopressin intranasally.
- Children 1 – 5 years: 2.5 – 5 µg Desmopressin intranasally.
- 5 years: 5 – 10µg Desmopressin intranasally.
- ***As alternative, Desmopressin may be administered by injection: dose 0.5 µg/m^2 BSA (approximately 10 x potency of nasal).***

Sample	Baseline 0 hours	Every 2 hours from time specified by medical officer	Every 1 hour as weight loss ap-proaches 5%	After DDAVP administration 4 hourly or as specified
Urine specific gravity (refractometer)	S	S	S	S

Urine osmolality	S	S	S	S
Plasma Na, K	S	S	S	S
Plasma osmolality	S	S	S	S
Plasma antidiuretic hormone (ADH)	S	In addition to baseline, collect ADH at end of test (before DDAVP) and one other time-point beyond the estimated half-way point of test		

Interpretation:

- Test can be properly interpreted only if plasma osmolarity greater than 290 is reached. At lower levels, ADH secretion is not maximally stimulated, urine may not be concentrated, and a response to pitressin may be present.
- Central diabetes insipidus (DI) is diagnosed when plasma osmolarity is elevated, urine osmolarity is low and there is a significant response to pitressin. In nephrogenic DI, there is no response to pitressin.
- In subjects without DI, urine volume drops, and urine osmolality increases usually to at least twice to three times plasma osmolality (age-dependent).
- Blood osmolality does not rise significantly. Plasma ADH levels rise. In subjects with ADH deficiency (central DI), urine losses continue, and dehydration ensues, plasma osmolality increases, with no or little rise in urine osmolality.
- In partial DI, urine osmolality may rise to a peak of 300 - 600 mosm/l. ADH rise may be poor, but this is not diagnostic.
- Administration of DDAVP leads to urinary concentration.
- In nephrogenic DI, findings are like ADH deficiency, except there is no or poor response to DDAVP administration, and ADH levels are usually clearly elevated.