Manchester University
NHS Foundation Trust

eDOCUMENT CONTROL PAGE

Title:	Endocrine Dynamic Function Test Protocols for use in Neonates & Children (Biochemistry Reference Number: BC-CL-PR-18)
Version:	8
Supersedes:	Version 7
Application:	**RMCH/MCS**

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Ratified by:	RMCH/MCS Policies and Guidelines Group (PGG) PMMC | March 24 | 2x medicine amendments reviewed by pharmacy. Note - document has not had a full PMMC/pharmacy review as no pharmacist in post. The remaining monographs should be prioritized when endocrine pharmacist next in post. **See minutes.**
Date of Ratification:	August 2024

Issue / Circulation Date:	August 2024
Circulated by:	RMCH/MCS Policies and Guidelines Group (PGG)
Dissemination and Implementation:	Available on the MFT Policy Hub
Date placed on the Intranet:	September 2024

Planned Review Date:	August 2026
Responsibility of:	Consultant Clinical Biochemist, Principal Clinical Biochemist, Consultant Paediatric Endocrinologist, Clinical Nurse Specialist

Minor Amendment (If applicable) Notified To:	
Date notified:	

EqIA Registration Number:	2024-22

Introduction

This document describes agreed protocols for endocrine dynamic function tests in neonates and children.

Purpose

Each protocol includes the principle of the test, indications, precautions, side-effects, preparation, procedure, samples required and timings, test interpretation and references.

Equality Impact Assessment

This Policy has been equality impact assessed by the author using the Trust's Equality Impact Assessment (EqIA) framework.

Consultation, Approval and Ratification Process

The policy document was sent to the following groups for consultation and review:

Paediatric Endocrinologists, RMCH
Biochemistry Department, MFT
RMCH/MCS Policies and Guidelines Group
Paediatric Medicines Management Committee

Dissemination

The policy is available on the MFT Biochemistry webpages. A link to this document is available on the trust policy hub.

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Endocrine Dynamic Function Test Protocols for use in Neonates and Children

Contents

Guidance

- Please read all protocols carefully to ensure that the lab is notified before any test which requires urgent analysis of specimens is commenced. If notification of the lab is required, this will be indicated at the start of the protocol.

- The time point from the dynamic function test protocol MUST be indicated on the blood sample tube.

- **In order to minimise haemolysis, a 22-gauge**, **blue cannula should be used to collect blood samples.**

- The volume of blood stated in these protocols assumes that there is a normal packed cell volume (PCV). If the child is known to have a high PCV, then please allow for this and send a larger volume of blood.

- Some of the blood samples required in these protocols need to arrive at the lab very urgently and on ice. Urgent porter delivery is arranged with Sodexo, who have a list of tests which have a porter response time of 5 min. These are termed 'Code Blue' tests. This form of words must be used when contacting the Sodexo helpdesk on x4850. In all cases the requesting clinician must contact the lab before the specimen is sent.

  - ACTH — 15 min on ice
  - Free fatty acids — 20 mins on ice

- Whilst some dynamic function tests may be requested via a specific test name on Hive, not all tests are set up in this way. If a test is being carried out which does not have an Hive test name, please order the analytes individually, indicating in the clinical details which test is being carried out.

Standard Dose Synacthen Test for Suspected Adrenal Failure

Test Name: CHILD SYNACTHEN DFT

Principle

Adrenal glucocorticoid secretion is controlled by adrenocorticotrophic hormone (ACTH) released by the anterior pituitary. This test evaluates the ability of the adrenal cortex to produce cortisol after stimulation by synthetic ACTH (tetracosactrin: Synacthen). The Synacthen test is a useful investigation in suspected secondary adrenal insufficiency as it correlates reasonably well with the 'gold-standard' insulin tolerance test but is safer and less unpleasant. Chronic ACTH deficiency results in adrenal atrophy which leads to a reduced response to exogenous ACTH. A home waking salivary cortisone has been shown to be accurate in diagnosing adrenal insufficiency with comparability to the Synacthen test.

Indication

- Screening test for suspected adrenal insufficiency.

Precautions

- The Synacthen test is unreliable if performed within 4 weeks of pituitary surgery as ACTH deficiency may not have been sufficiently prolonged to result in adrenal atrophy. An 8 - 9 am plasma ACTH and cortisol can be informative in these situations.
- The test is unreliable in patients taking the oral contraceptive pill. Any oral oestrogen therapy should be discontinued for 6 weeks prior to performing the synacthen test.
- Do not perform this test at the same time as an oral glucose tolerance test. However, the oral glucose tolerance may be performed after the synacthen test.

Side 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

Saliva Testing

On the day of the Synacthen test, a waking saliva sample is needed immediately after getting out of bed before brushing teeth, drinking/eating. Older children should be advised not to smoke or vape prior to the saliva sample.

Synacthen Testing

- The test should preferably be performed in the morning between 0800 and 0900 hrs.
- The patient does not need to be fasted once they have taken the saliva sample
- 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/m^2/day) prior to the test. Omit the dose the night before and on the morning of the test. If the paediatric 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.

Protocol

Saliva Testing

Patients are given Salivette tubes with a salivary collection swab or the longer SalivaBio swab with a standard 10 mL tube depending on their age. The Salivette are suitable for teenagers and the SalivaBio for younger children however, clinical judgement as to the competence of the individual child should be

used. Patients require written instructions/images on how to collect samples. On the day of the Synacthen test, a waking saliva sample is needed immediately after getting out of bed before brushing teeth, drinking/eating. Older children should be advised not to smoke or vape prior to the saliva sample.
If using a Salivette tube, the collection swab should be chewed for 2 mins before sealing in the plastic tube for sending to the lab. If using the SalivaBio swab, about 2 cm should be put in the mouth and held at the end by either the child or a parent/legal guardian/hospital staff member. It takes about 2 mins to saturate the swab, after which it can be removed, cut to size and place in a standard 10 cm tube for sending to the lab. A flowchart of the process can be found in appendix 1.

Synacthen Testing

A number of different protocols with different synacthen doses are available. We have taken a pragmatic approach, considering the ease of use.

1. Insert a reliable cannula and, if possible, rest the patient for 30 minutes.
2. Collect an ACTH sample at baseline (if requested)
3. Take basal blood sample for cortisol (t = 0).
4. ***Give Synacthen as an i.v. bolus***

Age	***Generic***	***Brand (if applicable)***	***Route***	***Dose***	***Frequency***	***Comments***
<1 month	*Tetracosatide*	*Synacthen*	*i.v*	*36 micrograms/kg*	*Bolus*	
1-12 months	*Tetracosatide*	*Synacthen*	*i.v*	*125 micrograms*	*Bolus*	*Use 36 micrograms/kg for preterm babies who remain in hospital.*
>1 year	*Tetracosatide*	*Synacthen*	*i.v*	*250 micrograms*	*Bolus*	

5. Take a blood sample at + 30 min after Synacthen for cortisol.

Samples

Cortisol 1.2 mL lithium heparin (orange top) or clotted blood (white top)

ACTH 1.8 mL EDTA tube (pink top)
Send IMMEDIATELY to laboratory on ice for centrifugation and freezing

Interpretation

Saliva Testing

• Waking salivary cortisone of <7nmol/L is consistent with adrenal insufficiency.
• Waking salivary cortisone of ≥17nmol/L is consistent with not being adrenal insufficient.
• Waking salivary cortisone levels between 7 and 16.9 nmol/L are equivocal and in these circumstances the Synacthen test result should be used on its own.

Synacthen Testing

Please note that synacthen test cut-offs vary from laboratory to laboratory and are dependent on the cortisol assay method. The MFT cut-off for both the Roche Gen II and LC-MS methods is 430 nmol/L. The table below displays cut-offs for other methods (El-Farhan et al., 2012) but please note new assay formulations which may require a change to the cut-off.

Method	SST cut-off
Siemens Centaur	446
Beckman Access	459
GC-MS	420
Architect	430
Immulite 2000	474

- A normal response is an increase in plasma/serum 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 dose of Synacthen used is supra-physiological and may give a normal response in patients with mild adrenal insufficiency.
- 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. A urine steroid profile may also be misleading after only 24 hours off hydrocortisone. The urine steroid lab at King's College Hospital recommends changing the glucocorticoid to dexamethasone and stimulating with depot Synacthen for up to 5 days before sample collection, unless glucocorticoid treatment has been brief. Please discuss with the paediatric endocrine team and the laboratory.

References

1. 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
2. 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.
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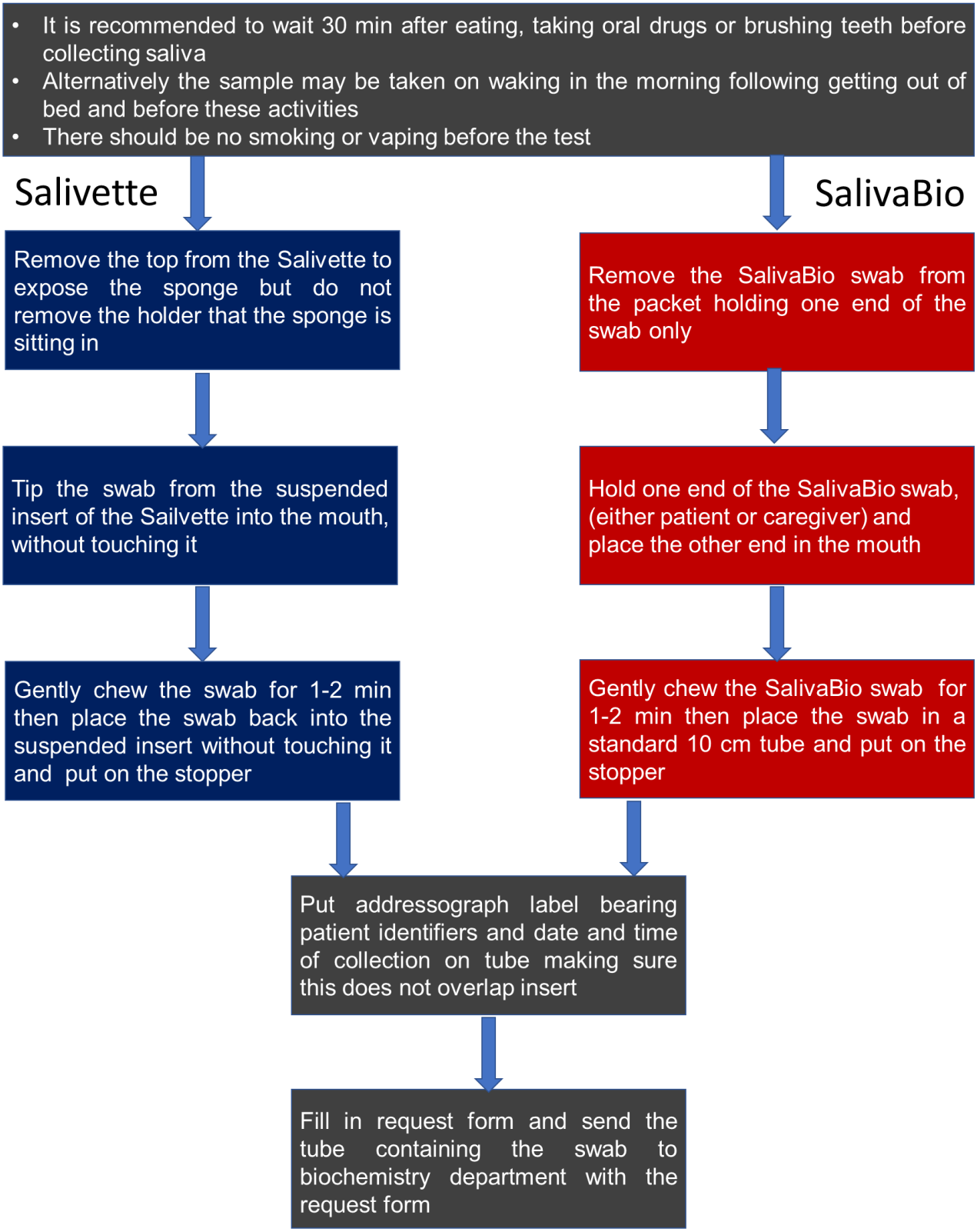
6. Debono M, Elder CJ, Lewis J, Fearnside J, Caunt S, Dixon S, Jacques RM, Newell-Price J, Whitaker MJ, Keevil B, Ross RJ. Home Waking Salivary Cortisone to Screen for Adrenal Insufficiency. NEJM Evidence, 2023, Jan 6.
7. Debono M, Caunt S, Elder C, Fearnside J, Lewis J, Keevil B, Dixon S, Ross R. Real world evidence supports waking salivary cortisone as a screening test for adrenal insufficiency. Clinical Endocrinology, 2023, 99: 517–524.

Appendix 1: Saliva Collection Flowchart



Standard Dose Synacthen Test for Congenital Adrenal Hyperplasia (CAH)

Test Name: CHILD SYNACTHEN & 17OHP DFT

Principle

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 CAH due to 21-hydroxylase deficiency in children and adults.

Precautions

- The Synacthen test gives unreliable results if performed within 4 weeks of pituitary surgery.
- Do not perform at the same time as an oral glucose tolerance test. However, the oral glucose tolerance may be performed after this test.

Side 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/m^2/day) prior to the test. Omit the dose the night before and on the morning of the test. If the paediatric 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.

Protocol

A number of different protocols with different synacthen doses are available. We have taken a pragmatic approach, considering the ease of use.

1. Insert a reliable cannula and, if possible, rest patient for 30 minutes.
2. Take basal blood sample for cortisol and 17-OHP (t = 0). Take baseline ACTH sample (if requested)
3. ***Give Synacthen as an i.v. bolus as per the table below:***

Age	Generic	Brand	Route	Dose	Frequency	Comments
<1 month	*Tetracosatide*	*Synacthen*	*i.v*	*36 micrograms/kg*	*Bolus*	
1-12 months	*Tetracosatide*	*Synacthen*	*i.v*	*125 micrograms*	*Bolus*	*Use 36 micrograms/kg for preterm babies who remain in hospital.*
>1 year	*Tetracosatide*	*Synacthen*	*i.v*	*250 micrograms*	*Bolus*	

4. Take blood samples at + 30 min after Synacthen (for cortisol and 17OHP)
+ 60 min after Synacthen. (for 17OHP only)

Samples

Cortisol 1.2 mL lithium heparin (orange top) or clotted blood (white top)

17–OHP 1.2 mL lithium heparin (orange top) or clotted blood (white top)

Interpretation

- Unaffected adults and 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 <9.8 nmol/L at 60 minutes.
- A stimulated 17-OHP between ≥9.8 but ≤30 nmol/L is an equivocal response and CAH is not excluded. Genotyping and/or a urine steroid profile is recommended.
- A stimulated 17-OHP of ≥30 nmol/L is consistent with a diagnosis of CAH. Genotyping of the 21-hydroxylase gene and urine steroid profiling can be used to confirm the diagnosis.
- Milder elevations of 17-OHP may be found in rarer forms of CAH: 11-β-hydroxylase deficiency and 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/serum cortisol to a level of ≥430 nmol/L at 30 minutes.

References

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Low Dose Synacthen Test

Test Name: CHILD LOW DOSE SYNACTHEN TEST DFT

Please note that this test is no longer commonly used.

Principle

Adrenal glucocorticoid secretion is controlled by adrenocorticotrophic hormone (ACTH) released by the anterior pituitary. This test evaluates the ability of the adrenal cortex to produce cortisol after stimulation by synthetic ACTH (tetracosactrin: Synacthen). The low-dose test is thought to be a more sensitive version of the standard dose Synacthen test, using a physiological rather than a pharmacological dose of Synacthen.

Indication

The low-dose test may be indicated in children who have a normal response to the standard dose Synacthen test, but a clinical history (e.g., chronic steroid therapy or symptoms, such as hypoglycaemia), suggestive of adrenocortical insufficiency. Use this low dose test for children who have been on inhaled or topical steroids, on corticosteroid treatment and when partial adrenal insufficiency is suspected.

Precautions

- The test is unreliable in patients taking the oral contraceptive pill.
- The dose of Synacthen involved in this test is very low. Great care must be taken with preparation and administration.
- Do not perform at the same time as an oral glucose tolerance test.

Side 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 patient does not need to be fasted.
- This test can be performed at any time of day
- 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/m^2/day) prior to the test. Omit the dose the night before and on the morning of the test. If the paediatric 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.

Protocol

1. Insert reliable cannula and rest patient for 30 minutes.
2. Prepare ***1 microgram solution of Tetracosatide from 250 micrograms vial*** as follows:
   - Dilute 1 mL to 50 mL with normal saline giving 250 micrograms in 50 mL
   - Take 1 mL of above solution and dilute with 9 mL of saline giving 5 micrograms in 10 mL.
   - The diluted dose must be freshly prepared.
3. Take basal blood sample for cortisol (t = 0 min).
4. Administer 2 mL of above solution (1 microgram) to patient i.v.
5. Flush the line with 5 mL saline to ensure that the whole dose has been administered.
6. Take blood samples at + 20 min
   + 30 min

+ 40 min
after Synacthen, for cortisol

Samples

Cortisol: 1.2 mL lithium heparin (orange top) or clotted blood (white top)

Interpretation

- A normal response is a peak cortisol level of ≥430 nmol/L. Levels below 430 nmol/L indicate a degree of adrenal insufficiency.

- In patients on long-term glucocorticoids, it is difficult to differentiate underlying adrenocortical disorders from the adrenal-suppressive effects of the treatment. A urine steroid profile may also be misleading after only 24 hours off hydrocortisone. The urine steroid lab at King's College Hospital recommends changing the glucocorticoid to dexamethasone and stimulating with depot Synacthen for up to 5 days before sample collection, unless glucocorticoid treatment has been brief. Please discuss with the paediatric endocrine team and the laboratory.

References

1. Elder C.J., Sachdev P. & Wright N.P. (2012) The short Synacthen test: a questionnaire survey of current usage. *Arch Dis Child* 97: 870-873
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Diagnosis & Differential Diagnosis of Cushing's Syndrome

Cushing's syndrome comprises a large group of signs and symptoms reflecting prolonged and inappropriately high exposure of tissues to glucocorticoids. Recent Endocrine Society clinical practice guidelines contain recommendations for the diagnosis of Cushing's syndrome.

Before commencing testing
Exclude the use of excessive exogenous glucocorticoids which may result in iatrogenic Cushing's syndrome before conducting biochemical testing.

Testing should be initiated in each of the following conditions:

- *Patients with unusual features for their age:*
  - *In children this includes those with decreasing height percentile and increasing weight*
- *Patients with multiple and progressive features, particularly those more predictive of Cushing's syndrome. In children this may include:*
  - *Slow growth/ short stature*
  - *Abnormal genital virilisation/ pseudoprecocious puberty or delayed puberty*
- *Patients with adrenal incidentaloma compatible with adenoma*

Initial testing for Cushing's syndrome should include one of the following tests:

- *Urine Free Cortisol (UFC; at least two measurement)*
- *Midnight salivary cortisol (two measurements)*
- *1-mg overnight dexamethasone suppression test (DST)*
- *Longer low dose DST (2 mg/day for 48 hrs)*

Definition of the cause of Cushing's syndrome should include the following tests:

- *9 am Plasma ACTH*
- *CRH Test*
- *Analysis of change in serum cortisol during Low dose dexamethasone suppression test*
- *Adrenal/Pituitary MRI scan*
- *Bilateral inferior petrosal sinus sampling for ACTH (with CRH)*

The following tests are not recommended in order to test for Cushing's syndrome:

- *Random serum cortisol or plasma ACTH levels*
- *Urinary 17-ketosteroids*
- *Insulin tolerance test*
- *Loperamide test*
- *Tests designed to determine the cause of Cushing's syndrome (e.g., pituitary and adrenal imaging, 8 mg DST)*

- Abnormal initial test results should be further investigated using a second recommended test.
- Further testing for Cushing's syndrome of individuals with concordantly negative results on two different tests is not recommended.
- Patients with concordantly positive results from two different tests should be further tested to establish the cause of Cushing's syndrome, provided there is no concern regarding possible non-Cushing's hypercortisolism.
- Further evaluation of patients with concordantly negative results may be appropriate in patients suspected of having cyclical disease, especially if the pre-test probability of Cushing's syndrome is high.

References

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2. Savage M.O., Chan L. F., Grossman A.B. & Storr H.L. (2008) Work up and management of Paediatric Cushing's Syndrome. *Curr Opin Endocrinol Diabetes Obes* **15**(4):346-51

Low Dose Dexamethasone Suppression Test

Test Name: CHILD LOW DOSE DEXAMETHASONE SUPPRESSION TEST

Principle

Cushing's syndrome comprises a large group of signs and symptoms which are the result of prolonged exposure to inappropriately high levels of glucocorticoids. In normal subjects the administration of a supra-physiological dose of glucocorticoid results in suppression of ACTH and cortisol secretion. In endogenous Cushing's syndrome of any cause, there is a failure of this suppression when a low dose of the synthetic glucocorticoid dexamethasone is given. The low dose dexamethasone suppression test has been reported to have a sensitivity and specificity of 94% when used to differentiate paediatric patients with Cushing's syndrome from normal individuals. This 48-hour 2 mg/day low dose protocol has improved specificity compared to the overnight test.

Indication

- To diagnose Cushing's syndrome

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 also falsely elevate cortisol results including oestrogens.
- Dexamethasone clearance maybe reduced in patients with liver and/ or renal failure.
- Dexamethasone should be used cautiously in a child with diabetes mellitus with meticulous measurements of blood glucose during the period of the test.
- The child should not be on exogenous glucocorticoids during the test including steroid creams, inhalers and eye drops.

Side Effects

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

Preparation

- None required.

Protocol

ACTH samples should be sent IMMEDIATELY to laboratory on ice for centrifugation and freezing

1. **Day 1** - Take blood samples for cortisol and plasma ACTH at 0900h and 2400h
2. **Days 2 and 3** - Starting at 0900h administer dexamethasone every 6 hours (i.e., 1500, 2100, 0300h) as follows:

   a. If the patient weighs more than 40 kg, use a dose of 0.5 mg dexamethasone
   b. If the patient weighs less than 40 kg, adjust the dose to 30 micrograms/kg/day (divided into 4 daily doses)

   All doses must be adhered to for the test to be valid.
3. **Day 4** - Take blood samples for serum cortisol and plasma ACTH at 0900h, 6 hr after the last dose of dexamethasone.

Time Points:

Day	Time (h)	Procedure	Sample
1	0900	-	Blood for Cortisol/ ACTH
	2400	-	Blood for Cortisol/ ACTH
2	0900	0.5 mg Oral Dexamethasone	-
	1500	0.5 mg Oral Dexamethasone	-
	2100	0.5 mg Oral Dexamethasone	-
3	0300	0.5 mg Oral Dexamethasone	-
	0900	0.5 mg Oral Dexamethasone	-
	1500	0.5 mg Oral Dexamethasone	-
	2100	0.5 mg Oral Dexamethasone	-
4	0300	0.5 mg Oral Dexamethasone	-
	0900	-	Blood for Cortisol/ ACTH

Samples

ACTH 1.8 mL EDTA tube (pink top)

Send IMMEDIATELY to laboratory on ice for centrifugation and freezing

Cortisol 1.2 mL lithium heparin (orange top) or clotted blood (white top)

Interpretation

- If the cortisol result on day 3 is <50 nmol/L, the patient has shown appropriate suppression and Cushing's syndrome can be ruled out.
- Patients with Cushing's syndrome, from whatever cause, lose the normal negative feedback control by circulating glucocorticoids on ACTH release and thus exhibit detectable plasma ACTH and cortisol concentrations after dexamethasone administration.
- In patients who fail to suppress, a pre-test ACTH level of <5 ng/L is highly suggestive of an adrenal cause of Cushing's syndrome.
- Cortisol suppression >30% following the low dose dexamethasone suppression test correlates well with the response in the high dose dexamethasone suppression test and is therefore suggestive of Cushing's disease.

References

1. Nieman L.K., Beverly M.K.B., Findling 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
2. 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 paediatric Cushing's syndrome. *Horm Res* **65**(3): 159 - 162

High Dose Dexamethasone Suppression Test

Test Name: CHILD HIGH DOSE DEXAMETHASONE SUPPRESSION TEST

Principle

This test is used in patients who have Cushing's syndrome established by screening, but with requirement for the aetiology to be further identified. The test works on the basis that in most situations the corticotroph tumour cells in Cushing's disease retain some responsiveness to the negative feedback of glucocorticoids, whilst tumours 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.

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 also falsely elevate cortisol results including oestrogens.
- Dexamethasone clearance maybe reduced in patients with liver and/ or renal failure.
- Dexamethasone should be used cautiously in a child with diabetes mellitus with meticulous measurements of blood glucose during the period of the test.
- The child should not be on exogenous glucocorticoids during the test including steroid creams, inhalers and eye drops.

Side Effects

- No significant side effects.

Preparation

- This test may be performed sequentially following the LDDST.

Protocol

ACTH samples should be sent IMMEDIATELY to laboratory on ice for centrifugation and freezing

1. **Day 1** - Take blood samples for cortisol and plasma ACTH at 0900h and 2400h
2. **Days 2 and 3** - Starting at 0900h administer dexamethasone every 6 hours (i.e. 1500, 2100, 0300h) as follows:

    a. If the patient weighs more than 40 kg use a dose of 2 mg dexamethasone
    b. If the patient weighs less than 40 kg use a dose of 120 micrograms/kg/day (divided into 4 daily doses)

    All doses must be adhered to for the test to be valid.
3. **Day 4** - Take blood sample for serum cortisol and plasma ACTH at 0900h, 6 hr after the last dose of dexamethasone.

Time Points:

Day	Time (h)	Procedure	Sample
1	0900	-	Blood for Cortisol/ ACTH
	2400	-	Blood for Cortisol/ ACTH
2	0900	Oral Dexamethasone	-
	1500	Oral Dexamethasone	-
	2100	Oral Dexamethasone	-
3	0300	Oral Dexamethasone	-
	0900	Oral Dexamethasone	-
	1500	Oral Dexamethasone	-
	2100	Oral Dexamethasone	-
4	0300	Oral Dexamethasone	-
	0900	-	Blood for Cortisol/ ACTH

Samples

ACTH 1.8 mL EDTA tube (pink top)
Send IMMEDIATELY to laboratory on ice for centrifugation and freezing

Cortisol 1.2 mL lithium heparin (orange top) or clotted blood (white top)

Interpretation

- Patients with pituitary-dependent hypercortisolism (Cushing's disease) will usually show suppression of plasma cortisol to at least 50% of basal values. Those with ectopic ACTH secretion will not show any suppression of Cortisol. Please note that approximately 10% of patients with Cushing's disease fail to suppress and approximately 10% of those with ectopic ACTH secretion will suppress.

References

1. Nieman L.K., Beverly M.K.B., Findling 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

CRH Test

Test Name: CHILD CRH STIMULATION DFT

Principle

CRH is normally released by the hypothalamus to stimulate ACTH release by the anterior pituitary. The administration of a CRH analogue (corticorelin) can therefore be used to assess the ability of the pituitary gland to secrete ACTH for the stimulation of cortisol production. Generally, patients with pituitary ACTH deficiency have a decreased ACTH and cortisol response to CRH. Patients with hypothalamic disorders however have an exaggerated and prolonged plasma ACTH response and a subnormal cortisol response.

The CRH test may also be used in combination with dexamethasone suppression tests for the differential diagnosis of Cushing's syndrome. CRH administration results in an excessive rise in plasma ACTH and serum cortisol in patients with pituitary Cushing's disease, whilst this is rarely seen in patients with ectopic ACTH secretion. The CRH test can therefore be used in the differential diagnosis of Cushing's syndrome to confirm whether the cause is pituitary-dependent or ectopic.

Indication

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

Precautions

- Imipramine may reduce the ACTH response.

Side Effects

- Flushing of the face, neck and upper body, hypotension or a mild sensation of taste or smell may occur following administration of CRH.

Preparation

- The patient should be fasted overnight (for a minimum of 4 hours).
- The patient should remain supine throughout the test.
- If the patient is to also have a high dose dexamethasone suppression test, the CRH test should be performed first.

Protocol

1. Insert a reliable cannula and wait 30 minutes before proceeding with the test.
2. Take a blood sample for ACTH and cortisol 15 minutes after the insertion of the cannula.
3. 15 min later, *administer CRH:*

Generic	***Route***	***Dose***	***Frequency***
Corticorelin	*i.v*	*1 microgram/kg body weight (to a maximum of 100 micrograms), to be administered over 30 seconds.*	*Bolus*

   Collect blood samples for ACTH and cortisol (t = 0).

4. Take further blood samples for ACTH and cortisol at 15, 30, 45, 60, 90 and 120 min post CRH administration.

Time Points:

Time post CRH (min)	Procedure	Sample
-15	-	Blood for Cortisol/ ACTH
0	CRH administration	Blood for Cortisol/ ACTH
15	-	Blood for Cortisol/ ACTH
30	-	Blood for Cortisol/ ACTH
45	-	Blood for Cortisol/ ACTH
60	-	Blood for Cortisol/ ACTH
90	-	Blood for Cortisol/ ACTH
120	-	Blood for Cortisol/ ACTH

Samples

ACTH 1.8 mL EDTA tube (pink top)
Send IMMEDIATELY to laboratory on ice for centrifugation and freezing

Cortisol 1.2 mL lithium heparin (orange top) or clotted blood (white top)

Interpretation

- A peak increment of serum cortisol >20% and plasma ACTH >50% suggests Cushing's disease. The CRH test has a sensitivity of 86-93% and a specificity of 90-100% using these cut off values to discriminate Cushing's disease from ectopic ACTH secretion.
- A rise in ACTH by 35% at 15 and 30 min compared to basal levels also suggests a pituitary source.
- The CRH test has been reported to show a high sensitivity in diagnosis of Cushing's disease in pre-pubertal children.
- CRH is also used to aid bilateral petrosal sinus sampling. The diagnostic sensitivity of basal central/peripheral ACTH ratio >2 and >3 post CRH is 94%.

References

1. Nieman LK, Lacroix A, Martin KA. Corticotrophin-releasing hormone stimulation test. UpToDate April 2012

Cortisol Day Curve – Monitoring hydrocortisone replacement

Test Name: CHILD CORTISOL DAY CURVE – MONITORING HYDROCORTISONE REPLACEMENT DFT

Indication

- Patients with adrenal insufficiency require monitoring to ensure that they are receiving an adequate dose of hydrocortisone replacement therapy.

Precautions

- None

Preparation

- The patient is required to attend the ETC as a day case, generally arriving ***before*** taking their normal morning dose of hydrocortisone.
- In this situation, the baseline blood sample should be taken ***before*** the morning dose of hydrocortisone.
- Dependent on hospital admission, this protocol can be started at any time of day as long as it covers a number of hydrocortisone doses with sampling every 2 hours. The protocol indicated below is for guidance but may be adapted.

Protocol

1. Insert a reliable cannula in order to collect blood samples at the time points indicated or specified by the requesting clinician.

2. The time points of blood collection will depend upon the hydrocortisone treatment regimen of the patient. Blood samples for cortisol should be collected pre-dose and then 2 hourly, stopping with the next pre-dose blood sample. ACTH may also be measured every 4 hours if this is thought to be clinically useful. The table below is for guidance.

3. **All hydrocortisone doses should be prescribed and administered at exactly the same times as the patient would administer doses at home.**

4. If it is felt there is clinical need to continue to monitor the adequacy of hydrocortisone replacement, this profile can be continued with blood samples for cortisol every 2 hours and for ACTH every 4 hours.

Time Points:

Patients should take their normal dose of hydrocortisone at their usual times. Blood samples should be collected before the next hydrocortisone dose. This information should be written in the day curve template and stored with the patient notes.

Time	Time point	Sample
On patient arrival	0 hrs - Take blood sample before hydrocortisone dose	Blood for Cortisol/ACTH
08:00	Patient should take normal morning dose of hydrocortisone	
10:00	2 hrs	Blood for Cortisol
12:00	4 hrs	Blood for Cortisol/(ACTH)
	Patient should take normal lunchtime dose of hydrocortisone	

14:00	6 hrs	Blood for Cortisol
16:00	8 hrs	Blood for Cortisol/(ACTH)
18:00	10 hrs	Blood for Cortisol

Samples

Cortisol 1.2 mL lithium heparin (orange top) or clotted blood (white brown top)

ACTH 1.8mL EDTA tube (pink top)

Send ACTH samples IMMEDIATELY to laboratory on ice for centrifugation and freezing

Example Cortisol Day Curve Template

Date: *1st May 2020*

Name: *Txxxxx Rxxxx* Date of Birth: *01/01/2008* Hospital Number: *xxxxxx*

IMPORTANT – please remember to take blood samples for cortisol/ACTH **prior** to giving morning dose.

Normal times/dose – *10/5/5 mg hydrocortisone given @ 8am, 12noon, 6pm.*

Please fill in chart below with times that cortisol samples have been taken as well as the time and dose of any medication given.

Time medication given (include dose)	Time cortisol/ACTH samples were taken	Result
	08:05	*244 nmol/L*
08:10 am *10mg hydrocortisone (after blood sample)*		
	10:05	*511 nmol/L*
	12:10	*230 nmol/L*
12:15 *5 mg hydrocortisone*		
	14:00	*404 nmol/L*
	16:00	*131 nmol/L*

Cortisol Day Curve Template

Date:

Name: Date of Birth: Hospital Number:

IMPORTANT – please remember to take blood samples for cortisol/ACTH **prior** to giving morning dose.

Normal dosage regimen (times/dose) -

Please fill in chart below with times that cortisol samples have been taken as well as the time and dose of any medication given.

Time medication given (include dose)	Time cortisol/ACTH samples were taken	Result
	A **pre-dose** sample is always required	

Cortisol Day Curve – Assessment of adrenal function

Test Name: CHILD CORTISOL DAY CURVE – ASSESSMENT OF ADRENAL FUNCTION DFT

Indication

- Monitoring endogenous secretion of cortisol and ACTH may be rarely used in the assessment of adrenal function in order to further support the diagnostic work up.
- Patients with exogenous suppression of ACTH are evaluated for recovery of the hypothalamic-pituitary-adrenal axis by monitoring endogenous secretion of cortisol and ACTH during a period of time when patients have stopped taking corticosteroids.

Precautions

- None

Preparation

- For assessment of recovery of the hypothalamic-pituitary-adrenal axis steroid therapy should be stopped. 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/m^2/day) prior to the test. Omit the dose the night before and on the morning of the test. However, the patient should take their usual dose of corticosteroid as soon as the test is completed.

Protocol

1. Insert a reliable cannula in order to collect blood samples at the time points indicated.

2. Blood samples for cortisol should be collected 2 hourly in order to assess the diurnal variation of secretion. ACTH samples should be collected every 4 hours. The table below is for guidance.

3. If it is felt there is clinical need to continue to monitor adrenal function this profile can be continued as indicated with blood samples for cortisol every 2 hours and for ACTH every 4 hours

Time Points:

Time	Time point	Sample
08:00 or on patient arrival	Baseline	Blood for Cortisol/ACTH
10:00	2 hrs	Blood for Cortisol
12:00	4 hrs	Blood for Cortisol/ACTH
14:00	6 hrs	Blood for Cortisol

Samples

Cortisol 1.2 mL lithium heparin (orange top) or clotted blood (white top)

ACTH 1.8 mL EDTA tube (pink top)
Send IMMEDIATELY to laboratory on ice for centrifugation and freezing

Cortisol Day and Night Curve

Test Names: CHILD CORTISOL DAY AND NIGHT CURVE **NON-CAH** DFT or CHILD CORTISOL DAY AND NIGHT CURVE **CAH** DFT

Indication

- Patients with congenital adrenal hyperplasia (CAH) require monitoring to ensure that they are receiving adequate doses of hydrocortisone replacement therapy. The endocrine team at RMCH advocate a 24-hour cortisol curve for CAH patients. A 24-hour cortisol curve may also be appropriate for monitoring corticosteroid therapy for adrenal insufficiency.

Precautions

- None

Preparation

- The patient is required to attend the ETC overnight, continuing to take hydrocortisone as prescribed.

Protocol

1. Insert a reliable cannula in order to collect blood samples at the time points indicated.

2. The time points of blood collection will depend upon the hydrocortisone treatment regimen of the patient. Blood samples for cortisol should be collected pre-dose and then 2 hourly during the day and 4-hourly overnight. In patients with CAH, it may also be useful to collect blood samples for 17-α-hydroxyprogesterone at the same time points. Samples should be collected over a 24-hour period as indicated. The table below is for guidance.

3. All ***hydrocortisone doses should be prescribed and administered at exactly the same times as the patient would administer doses at home***. Blood samples should be collected before the next hydrocortisone dose. This information should be written in the day and night curve template and stored with the patient notes.

Time Points: *17OHP is only required in patients with CAH.*

Time (min)	Time point	Sample
16:00	Patient arrival on ward, take initial blood sample	Blood for Cortisol, 17OHP
18:00	2 hrs	Blood for Cortisol, 17OHP
	Patient should take normal night time dose of hydrocortisone	
22:00	6 hrs	Blood for Cortisol, 17OHP
2:00	10 hrs	Blood for Cortisol, 17OHP
6:00	14 hrs	Blood for Cortisol, 17OHP
8:00	16 hours	Blood for Cortisol, 17OHP
	Patient should take normal dose of hydrocortisone	
10:00	18 hrs	Blood for Cortisol, 17OHP
12:00	20 hrs	Blood for Cortisol, 17OHP
14:00	22 hrs	Blood for Cortisol, 17OHP
	Patient should take normal dose of hydrocortisone	

Samples

Cortisol 1.2 mL lithium heparin (orange top) or clotted blood (white top)

17–OHP 1.2 mL lithium heparin (orange top) or clotted blood (white top)

Cortisol Day and Night Curve Template

Date:

Name: Date of Birth: Hospital Number:

IMPORTANT – please remember to take blood samples for cortisol/ACTH **prior** to giving morning dose.

Normal dosage regimen (times/dose) -

Please fill in chart below with times that cortisol samples have been taken as well as the time and dose of any medication given.

Time medication given (include dose)	Time cortisol/ACTH samples were taken	Result
	A **pre-dose** sample is always required	

Diagnosis of Growth hormone deficiency (GHD) in children

If the patient is likely to become hypoglycaemic during GHD testing (due to a known issue with hypoglycaemia) a special individualised plan should be implemented prior to GHD testing.

The diagnosis of GHD in childhood is a multi-faceted process requiring clinical and auxological assessment, combined with biochemical tests of the GH-insulin-like growth factor (IGF) axis and radiological evaluation. Diagnosis can prove extremely difficult due to the poor reproducibility, specificity and sensitivity of the non-physiological biochemical tests involved. GHD may present as an isolated problem or in combination with multiple pituitary hormone deficiency (MPHD).

Evaluation for GHD in short stature (defined as height ≥2 s.d. below the population mean) should not be initiated until other chronic, non-endocrine causes of growth failure (e.g. hypothyroidism, chronic systemic disease, Turner's syndrome, skeletal disorder) have been excluded. Due to the intrinsic diagnostic inaccuracy of any GH provocation test, correct selection of the child to be tested remains of upmost importance.

Criteria to initiate investigation for GHD include:

1. *Severe short stature, defined as a height >3 s.d. below the population mean.*
2. Height >1.5 s.d. below the mid-parental height
3. Height >2 s.d. below the mean and a height velocity over 1 yr >1 s.d. below the mean for chronological age, or a decrease in height s.d. of >0.5 over 1 yr in children over 2 yrs of age.
4. *In the absence of short stature, growth failure as suggested by:*
    a. *A height velocity >2 s.d. below the mean over 1 year.*
    b. *A height velocity >1.5 s.d. below the mean sustained over 2 years.*
5. Signs indicative of an intracranial lesion
6. Signs of multiple pituitary hormone deficiency
7. Neonatal symptoms and signs of Growth hormone deficiency (e.g., in children with pituitary tumours, septo-optic dysplasia and neonatal hypoglycaemia)

The symptoms most commonly encountered are highlighted in italics.

Biochemical assessment of GHD

Random single GH estimations are rarely helpful in diagnosing or excluding GHD due to the pulsatile release of the hormone. Instead, a variety of provocation tests may be used, each following an overnight fast. A basic requirement is for diagnosis to be supported by at least 2 stimulation tests[4], and this requirement also been recommended by NICE[2]. The stimulation tests in current use are not ideal due to poor reproducibility and dependence on a number of factors such as body composition and pubertal status.

A peak plasma GH concentration of ≥7 µg/L indicates a normal response to the test and no further investigations are required. A peak plasma GH concentration of <5 µg/L is diagnostic of growth hormone deficiency. A peak plasma GH concentration of 5 – 7 µg/L may still be indicative of GH deficiency and requires further investigation.

In the transition from childhood to adulthood a peak GH concentration of <5 µg/L is used to determine patients requiring treatment. In adults a peak GH concentration of <3 µg/L is used to diagnose GHD.

The cut off levels used are arbitrary values as even normal children can have low peak GH values. The cut off value may be used independently of the type of test and assay methodology involved. This does, however, make interpretation difficult as it is well known that there is not only considerable inter and intra-

individual variation with these tests, but the GH response also varies depending on the assay and stimulus used.

Measurement of IGF-1 and IGFBP-3 are reflective of the circulating level of GH but have relatively small variation during the course of the day and as such can be used to aid discrimination between normal and abnormal GH release. For IGF-1 ranges, standardized for age and sex (see table on page 20), values below the reference range for age support an abnormality in the GH axis if other causes of low IGF (e.g., malnutrition) have been excluded. Nevertheless, in GHD values of IGF-1 and IGFBP-3 within the normal range can occur.

All GH provocation tests should be performed on a dedicated clinical investigation unit. On the Elective Treatment Centre (Ward 76) at RMCH, Arginine and Glucagon are the main provocation tests used.

IGF-1 Reference Ranges according to age, gender and tanner stage using the IDS iSYS IGF-1 assay (µg/L). Please note that these reference ranges are not applicable to other IGF-1 methods.

Age (in years unless specified)	Male	Female		Age (in years unless specified)	Male	Female
0-3 mths	27 - 157	18 - 126		11.5	119 - 477	99 - 477
4-6 mths	28 - 159	18 - 127		12	126 - 499	105 - 499
7-9 mths	28 - 161	19 - 128		12.5	133 - 517	111 - 518
10-12 mths	29 - 164	19 - 130		13	139 - 533	116 - 533
1	30 - 167	20 - 132		13.5	144 - 544	120 - 545
1.5	32 - 175	21 - 138		14	148 - 551	123 - 552
2	34 - 184	22 - 145		14.5	150 - 554	126 - 555
2.5	36 - 194	24 - 154		15	152 - 554	127 - 554
3	39 - 205	26 - 164		15.5	153 - 549	128 - 550
3.5	42 - 215	28 - 175		16	153 - 542	128 - 542
4	44 - 225	31 - 188		16.5	152 - 532	127 - 531
4.5	47 - 235	33 - 201		17	151 - 521	125 - 517
5	50 - 246	36 - 214		17.5	149 - 508	123 - 502
5.5	53 - 256	39 - 227		18 - 20	129 - 494	105 - 486
6	56 - 267	42 - 240		21 - 25	103 - 398	82 - 383
6.5	60 - 279	45 - 254		26 - 30	93 - 297	75 - 284
7	63 - 292	49 - 270		31 - 35	86 - 254	72 - 249
7.5	68 - 307	53 - 286		36 - 40	79 - 236	65 - 233
8	72 - 323	57 - 305		41 - 45	71 - 221	59 - 210
8.5	78 - 341	62 - 326		46 - 50	63 - 208	55 - 197
9	84 - 362	67 - 349		51 - 60	52 - 201	44 - 191
9.5	90 - 384	73 - 374		61 - 70	43 - 190	38 - 171
10	97 - 407	80 - 400		71 - 80	35 - 182	35 - 168
10.5	104 - 431	86 - 427		> 80	32 - 172	32 - 178
11	112 - 454	93 - 453				

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

Sex Steroid Priming

In pre- and peri-pubertal children who have a sub-normal response to provocative testing, sex steroid priming may increase the response to that seen in late puberty and should therefore be considered. Sex steroid priming can provide an adequate response to stimulation in healthy pre-pubertal children and is proposed to reduce the number of false positive test results. As a guideline, priming may be indicated in girls above 8 years, and boys above 9 years of age. There is currently no consensus regarding sex steroid priming prior to GH provocation testing[3], however the following protocol has been agreed by endocrinologists at RMCH. GH provocation tests following oestradiol priming have been reported to have the highest diagnostic accuracy[5].

GIRLS: Over 8 years of age, with no signs of puberty.

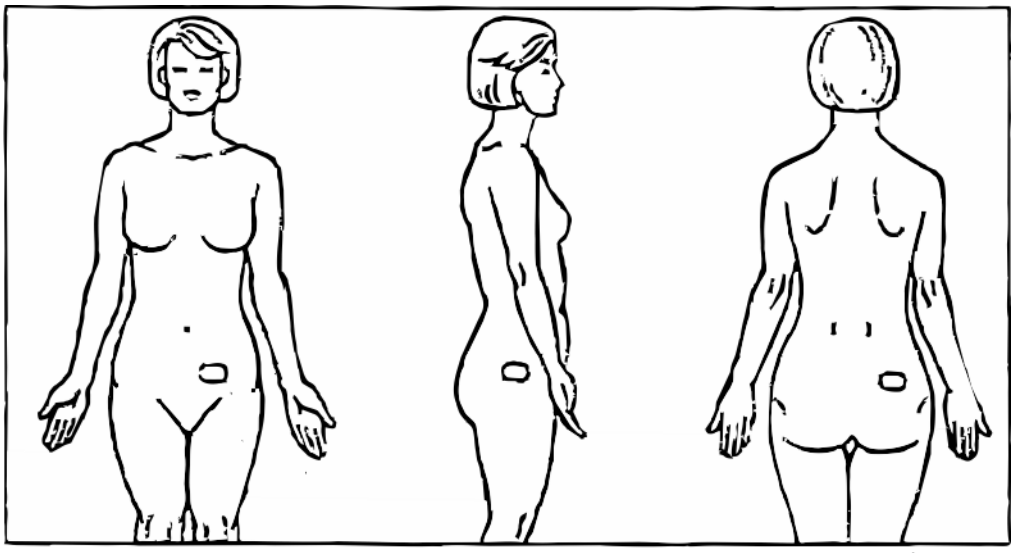
- Give Evorel/Estradot/EstradermMX 25 microgram patch to be applied 3 days before the test. If the patch is dislodged the clinician will decide if sufficient exposure to oestrogen has occurred to justify performing the test. Commence test on 4th day.
- Alternatively give 10-20 micrograms of oral ethinyl estradiol in the evening, daily, for 3 days prior to test.

BOYS: Over 9 years of age, with no signs of puberty.

- Give Evorel/Estradot/EstradermMX 25 microgram patch to be applied 3 days before the test. If the patch is dislodged the clinician will decide if sufficient exposure to oestrogen has occurred to justify performing the test. Commence test on 4th day.
- Alternatively give 10-20 micrograms of oral ethinyl estradiol in the evening, daily, for 3 days prior to test.
- Alternatively, 100 mg i.m testosterone esters (Sustanon) can be given 1 week prior to the test.

Instructions for using Evorel/Estradot/EstradermMX patches:

1. Cut the patch to size – patch should be 25 micrograms.
2. Peel of the backing and apply to an area highlighted in the diagram on next page.
3. If the patch falls off, re-apply and use Mepore dressing over the patch.
4. The patch can be used whilst showering.



References:

1. GH Research society (2000) JPEM 14: 377 – 382
2. Binder G. (2011) Growth hormone deficiency: new approaches to the diagnosis. Pediatr Endocrinol Rev 9 (Suppl 1): 535 - 537
3. Kumaran A. & Dattani M. (2008) Growth hormone deficiency – Difficulties in Diagnosis and Management. *Touch Briefings*
4. Stanley T. (2012) Diagnosis of Growth Hormone Deficiency in childhood. *Curr Opin Endocrinol Diabetes Obes* **19**:47-52
5. Petersenn S., Quabbe H.J., Schöfl C., Stalla G.K., von Werder K. & Buchfelder M. (2010) The Rational Use of Pituitary Stimulation Tests. *Dtsch Arztebl Int* **107**(25):437-43

Arginine Stimulation Test

Test Name: CHILD ARGININE STIMULATION DFT

Principle

Arginine is used as a provocative agent during a stimulation test in the diagnosis of children with suboptimal growth. Arginine reduces somatostatin release and stimulates α-adrenergic receptors resulting in GHRH release. The test has a sensitivity of 75% with a specificity of 85% using a diagnostic cut-off of 7 μg/L[1]. This can be increased to a sensitivity of 100% and specificity of 98% if clinical evidence of GHD is also present[2].

Indication

- See ***Diagnosis of Growth Hormone Deficiency***

Precautions

- None

Side Effects

- Arginine may cause nausea and some irritation at the infusion site, ensure that the cannula works well prior to arginine administration. **Arginine can cause chemical burn injury if administered incorrectly**. Administer the infusion of arginine over 15 min time period.
- Arginine may also rarely cause anaphylaxis.
- In children with suspected hypopituitarism prolonged fasting may induce hypoglycaemia. Blood glucose should be checked by POCT in these patients whenever a sample is taken.

Preparation

- Thyroid function should be normal; this must be ascertained before commencing the test.
- GH should be stopped for at least 4 weeks prior to the test.
- Sex steroid priming may be necessary, see ***Diagnosis of Growth Hormone Deficiency***
- Patients should have water only for 8 hours prior to the test.
- For very young children, particularly those <1 year of age, a smaller duration of fast, possibly 4 hours should be adequate. This should be discussed with the consultant endocrinologist.
- A small amount of water may be swallowed during the test (no i.v).

Protocol

1. Insert an indwelling cannula and take a basal blood sample (t= -30). Cannulation may cause growth hormone to rise; therefore, the patient should rest for 30 min before the test is commenced.

2. If the blood glucose meter <2.6 mmol/l at the start if the test, take a sample for glucose and growth hormone before administering glucose. DO NOT PROCEED WITH TEST – discuss with endocrine team first. It may be necessary to administer 10% glucose bolus.

3. If hypoglycaemia occurs during the test (blood glucose meter < 2.6 mmol/L administer 10 % glucose 2ml/Kg throughout the rest of the test.

4. Take a blood sample before commencing the infusion of arginine (t = 0).

Generic	***Route***	***Dose***	***Frequency***
L-arginine monohydrochloride (10% solution in 0.9% sodium chloride)	*i.v*	*0.5g/kg body weight up to a maximum of 30g*	*Infusion over 15 mins*

5. Take blood samples for growth hormone 15, 30, 45, 60, 90 and 120 min after **the start** of the arginine infusion (i.e., 15 min sample should be taken after the arginine infusion has been completed). At each time point also check the blood glucose of the patient using a blood glucose meter.

Time Points:

Time post arginine infusion (min)	Procedure	Blood Sample
-30	Check blood glucose using meter. Check ketones using meter if blood glucose <3.0 mmol/L.	Growth hormone
0	Check blood glucose using meter	Growth hormone
15	Check blood glucose using meter	Growth hormone
30	Check blood glucose using meter	Growth hormone
45	Check blood glucose using meter	Growth hormone
60	Check blood glucose using meter	Growth hormone
90	Check blood glucose using meter	Growth hormone
120	Check blood glucose using meter	Growth hormone

Samples

Growth Hormone 1.2 mL clotted blood (white top)

Interpretation

- A peak plasma GH concentration of ≥7 µg/L indicates a normal response to the test and no further investigations are required.
- A peak plasma GH concentration of <5 µg/L is diagnostic of growth hormone deficiency but requires a second GH provocation test to confirm.
- A peak plasma GH concentration of 5 – 7 µg/L may still be indicative of GH deficiency and requires further investigation.
- In adults, a peak plasma GH concentration of <3 µg/L is diagnostic of growth hormone deficiency.
- The percentage of children who are not GH deficient and who show a normal response to this test varies from 45 – 93%. Generally, 20% of normal children fail to respond to a formal test and this is the reason for doing 2 tests before proceeding to GH therapy. For example, 71% of normal individuals will respond to both insulin tolerance and arginine stimulation tests. However, the others will respond to at least one test: 13% to insulin, 16% to arginine.

References

1. Van Vught A.J.A.H., Nieuwenhuizen A.G., Gerver W.J., Veldhorst M.A.B., Brummer R.J.M. & Westerterp-Plantenga M.S. (2009) Pharmacological and Physiological Growth Hormone Stimulation. Tests to Predict Successful GH Therapy in Children. *JPEM* **22**:679 – 694
2. Binder G. (2011) Growth hormone deficiency: new approaches to the diagnosis. *Paediatric Endocrinol Rev* **9**(1): 535-537

Glucagon Stimulation Test for Growth Hormone

Test Name: CHILD GLUCAGON STIMULATION FOR GROWTH HORMONE DFT

Principle

This test is commonly used for the evaluation of growth hormone deficiency (GHD). Glucagon causes blood glucose to increase leading to insulin release and therefore indirectly stimulating GH and ACTH release through provocation of the hypothalamic-pituitary axis.

Indication

- See ***Diagnosis of Growth Hormone Deficiency***

Precautions

- The test should not be performed on a patient with phaeochromocytoma 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 disease. The inability to mobilise glycogen may result in hypoglycaemia.
- The test should not be carried out in patients with severe hypocortisolaemia (9 am level <100 nmol/L).
- Thyroid function must be normal as thyroxine deficiency may reduce the GH response.

Side Effects

- Nausea and abdominal pain are common (30%) and patients may rarely vomit.

Preparation

- Thyroid function and cortisol must be checked to rule out panhypopituitarism.
- GH should be stopped for at least 4 weeks prior to the test.
- Patients must fast for 8 hours prior to the test (only water is allowed).
- A small amount of water may be swallowed during the test.
- Sex steroid priming may be necessary, see ***Diagnosis of Growth Hormone Deficiency***

Protocol

Children can become hypoglycaemic after glucagon administration, usually 90 – 120 minutes post dose. Children <8 yrs of age are at particular risk. Check glucose levels (by glucose meter) at the time of every sample. 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 hypoglycaemia.

1. Insert an indwelling 22 gauge, blue, cannula and take a basal blood sample (t = -30). Wait 30 minutes before taking the baseline (t = 0) sample for growth hormone as cannulation may cause GH to rise.
2. Check glucose level by meter.
    - If glucose < 2.6 mmol/L at the start of the test - DO NOT PROCEED WITH TEST & DO NOT ADMINISTER GLUCAGON. Discuss with endocrine team. It may be necessary to administer 10% glucose 2 ml/kg throughout the test. Take a sample for glucose and growth hormone before administering glucose.
    - If glucose level > 2.6 mmol/L then administer ***glucagon:***

Generic	***Route***	***Dose***	***Frequency***
Glucagon	*i.m*	*30 micrograms/kg of body weight up to a maximum dose of 1 mg.*	*Bolus*

3. Take further blood samples for growth hormone at 60, 90, 120, 150 and 180 min post glucagon administration.
4. Observe for signs of hypoglycaemia throughout the test and record in patient's notes.
5. Remember to check the child's glucose level by meter and the responsiveness at every sample.

6. A sweet drink and a full meal must be eaten and tolerated after the test and the child should be observed for 1 hour after the test. Blood glucose (by meter) must be >4 mmol/L before discharge.

Time Points:

Time post glucagon (min)	Procedure	Blood Sample
-30	Check blood glucose using meter. Check ketones using meter if blood glucose <3.0 mmol/L.	Growth hormone
0	Check blood glucose using meter	Growth hormone
60	Check blood glucose using meter	Growth hormone
90	Check blood glucose using meter	Growth hormone
120	Check blood glucose using meter	Growth hormone
150	Check blood glucose using meter	Growth hormone
180	Check blood glucose using meter	Growth hormone

Samples

Growth Hormone 1.2 mL clotted blood (white top)

Interpretation

- A peak plasma GH concentration of ≥7 µg/L indicates a normal response to the test and no further investigations are required.
- A peak plasma GH concentration of <5 µg/L is diagnostic of growth hormone deficiency but requires a second GH provocation test to confirm the diagnosis.
- A peak plasma GH concentration of 5–7 µg/L may still be indicative of GH deficiency and requires further investigation.
- In adults, a peak plasma GH concentration of <3 µg/L is diagnostic of growth hormone deficiency.
- Peak GH responses are also highly dependent on both short-term nutritional status and on BMI – higher peak GH levels after short-term fasting and in those with lower BMI.

References

1. Basildon and Thurlow University Hospitals NHS Foundation Trust Clinical Biochemistry Department paediatric department Glucagon Stimulation test Paediatric protocol
2. Lim S.H., Vasanwala R., Lek N. and Yap F. (2011) Quantifying the risk of hypoglycaemia in children undergoing the glucagon stimulation test. *Clinical Endocrinology* **75**: 489 – 494
3. Strich D., Terespolsky N. and Gillis D. (2009) Glucagon stimulation test for childhood Growth Hormone deficiency: Timing of the peak is important. *The Journal of Pediatrics* 415 – 419
4. Secco A., di Iorgi N., Napoli F., Calandra E., Ghezzi M., Frassinetti C., Parodi S., Casini M.R., Lorini R., Loche S. and Maghnie M. (2009) The Glucagon Test in the diagnosis of growth hormone deficiency in children with short stature younger than 6 years. *JCEM* **94**(11): 4251-4257

Glucagon Stimulation Test for Cortisol & Growth Hormone

Test Name: CHILD GLUCAGON STIMULATION FOR CORTISOL AND GROWTH HORMONE DFT

Principle

This test can be used as an alternative to the insulin-induced hypoglycaemia test in the evaluation of central adrenal insufficiency. Glucagon requires endogenous ACTH to cause cortisol secretion.

Indication

- To identify secondary adrenal insufficiency or combined ACTH/GH deficiency

Precautions

- The test should not be performed on a patient with phaeochromocytoma 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 disease. The inability to mobilise glycogen may result in hypoglycaemia.
- The test should not be carried out in patients with severe hypocortisolaemia (9 am level <100 nmol/L)
- Thyroid function must be normal as thyroxine deficiency may reduce the GH and cortisol response.

Side Effects

- Nausea and abdominal pain are common (30%) and patients may rarely vomit.

Preparation

- Thyroid function and cortisol must be checked to rule out panhypopituitarism.
- GH should be stopped for at least 2 weeks prior to the test.
- 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/m^2/day) prior to the test. Omit the dose the night before and on the morning of the test. If the paediatric 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.
- Patients must fast for 8 hours prior to the test (water only is allowed).
- A small amount of water may be swallowed during the test.
- Sex steroid priming may be necessary, see ***Diagnosis of Growth Hormone Deficiency***

Protocol

Children can become hypoglycaemic after glucagon administration, usually 90 – 120 minutes post dose. Children <8 yrs of age are at particular risk. Check glucose levels (by glucose meter) at the time of every sample. 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 hypoglycaemia.

1. Insert an indwelling 22 gauge, blue, cannula and wait 30 minutes before taking the baseline (t=0) sample for cortisol and growth hormone.

2. Check glucose level by meter.
   - If glucose < 2.6 mmol/L at the start of the test - DO NOT PROCEED WITH TEST & DO NOT ADMINISTER GLUCAGON. Discuss with endocrine team. It may be necessary to administer 10% glucose 2 ml/kg throughout the test. Take a sample for glucose and growth hormone before administering glucose.
   - If glucose level >2.6 mmol/L then administer ***glucagon:***

Generic	*Route*	*Dose*	*Frequency*
Glucagon	*i.m*	*30 micrograms/kg of body weight up to a maximum dose of 1 mg.*	*Bolus*

3. Take further blood samples for cortisol at 60, 90, 120, 150 and 180 min post glucagon administration.
4. Observe for signs of hypoglycaemia throughout the test and record in patient's notes.
5. Remember to check the child's glucose level by meter and the responsiveness at every sample.
6. A sweet drink and a full meal must be eaten and tolerated after the test and the child should be observed for 1 hour after the test. Blood glucose (by meter) must be >4 mmol/L before discharge.

Time Points:

Time post glucagon (min)	Procedure	Blood Sample
-30	Check blood glucose using meter	Cortisol, Growth Hormone
0	Check blood glucose using meter	Cortisol, Growth Hormone
60	Check blood glucose using meter	Cortisol, Growth Hormone
90	Check blood glucose using meter	Cortisol, Growth Hormone
120	Check blood glucose using meter	Cortisol, Growth Hormone
150	Check blood glucose using meter	Cortisol, Growth Hormone
180	Check blood glucose using meter	Cortisol, Growth Hormone

Samples

Cortisol 1.2 mL lithium heparin (orange top) or clotted blood (white top)

Growth Hormone 1.2 mL clotted blood (white top)

Interpretation

- A peak plasma cortisol concentration of ≥430 nmol/L is indicative of a normal response and normal adrenal function.
- A peak plasma GH concentration of ≥7 µg/L indicates a normal response to the test and no further investigations are required.
- A peak plasma GH concentration of <5 µg/L is diagnostic of growth hormone deficiency but requires a second GH provocation test to confirm the diagnosis.
- A peak plasma GH concentration of 5–7 µg/L may still be indicative of GH deficiency and requires further investigation.
- In adults, a peak plasma GH concentration of <3 µg/L is diagnostic of growth hormone deficiency.
- Peak GH responses are also highly dependent on both short-term nutritional status and on BMI – higher peak GH levels after short-term fasting and in those with lower BMI.

References

1. Basildon and Thurlow University Hospitals NHS Foundation Trust Clinical Biochemistry Department paediatric department Glucagon Stimulation test Paediatric protocol
2. Lim S.H., Vasanwala R., Lek N. and Yap F. (2011) Quantifying the risk of hypoglycaemia in children undergoing the glucagon stimulation test. *Clinical Endocrinology* **75**: 489 – 494
3. Strich D., Terespolsky N. and Gillis D. (2009) Glucagon stimulation test for childhood Growth Hormone deficiency: Timing of the peak is important. *The Journal of Pediatrics* 415 – 419
4. Secco A., di Iorgi N., Napoli F., Calandra E., Ghezzi M., Frassinetti C., Parodi S., Casini M.R., Lorini R., Loche S. and Maghnie M. (2009) The Glucagon Test in the diagnosis of growth hormone deficiency in children with short stature younger than 6 years. *JCEM* **94**(11): 4251-4257

Insulin Tolerance Test

This test is potentially dangerous and is <u>not</u> carried out routinely at RMCH. Consult with the paediatric endocrine team at RMCH if you are considering undertaking this test. It should only be carried out in specialist centres by experienced staff.

Test Name: CHILD INSULIN TOLERANCE TEST DFT

Principle

The insulin tolerance test is the gold standard test for assessing the integrity of the hypothalamo-pituitary-adrenal axis. Stress, in this case hypoglycaemia, 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 hypoglycaemia which must be treated immediately if the symptoms become severe.

Indication

- This test is not routinely used at RMCH, although it is considered the gold standard test to assess the integrity of the hypothalamo-pituitary-adrenal axis. We are most likely to use the ITT when re-testing a young person for the presence of persistent GH deficiency at the end of growth. The test may also be required for some research protocols.

Precautions

- This test should not be carried out in a child with a history of epilepsy or cardiac arrhythmias.
- The test should be used with particular caution in young children as the symptoms of hypoglycaemia may be difficult to detect.
- This test should not be carried out on patients with severe panhypopituitarism or hypoadrenalism.
- This test should not be carried out in a patient with a glycogen storage disorder.
- A doctor must be present throughout this test with the patient being closely monitored for symptoms of hypoglycaemia which may require treatment.

Side Effects

- Sweating
- Palpitations
- Impaired or loss of consciousness

Preparation

- The patient must be fasted overnight (4 hours for infants), although drinks of water are allowed.
- Ensure that glucose (10% glucose) and hydrocortisone are available for i.v. injection if necessary.
- A glucose drink must be available. This may be ~40g glucose powder (4 heaped teaspoons) dissolved in approximately half a glass of squash, alternatively POLYCAL or rapilose can be administered.
- Child must remain on the ward and eat for at least an hour after the test before the cannula is removed and the patient discharged.

Protocol

Children can become severely hypoglycaemic after insulin administration. Check glucose levels (by glucose meter) at the time of every sample and observe the child continuously for symptoms of severe hypoglycaemia. 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 hypoglycaemia.

1. Start the test between 0800h and 0900h. Weigh the patient and insert an indwelling cannula and take a basal blood sample (t = -30) for glucose, growth hormone and cortisol. Wait 30 minutes before taking the baseline (t = 0) sample for glucose, growth hormone and cortisol as cannulation may cause GH to rise. The patient should be resting throughout the test.

2. Check glucose level by meter.

- If glucose <3.5 mmol/L do not administer insulin.
- If glucose level 3.5 – 4.5 mmol/L then administer ***half the dose of insulin***
- If glucose > 4.5 mmol/L then continue with the test as indicated

3. Dilute soluble insulin (Actrapid) with normal saline to give a solution containing 1 unit per ml. ***Give an i.v. dose of 0.1 units per kg body weight***

This dose should be reduced to 0.05 units per 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.

4. Monitor blood glucose closely until adequate hypoglycaemia has been established (<2.2 mmol/L) or the child shows signs of hypoglycaemia (e.g. sweating or drowsiness). Administer glucose drink of ~40g glucose powder (4 heaped teaspoons) dissolved in approximately half a glass of squash, or POLYCAL or rapilose can be administered. If there are more severe symptoms of hypoglycaemia (e.g., impaired consciousness), i.v. glucose may be required.

5. Take further blood samples for glucose, growth hormone and cortisol at 15, 30, 60 and 90 min post insulin administration

6. Remember to check the child's glucose level by meter and the responsiveness at every sample.

Time Points:

Time post insulin (min)	Procedure	Blood Sample
-30	Check blood glucose using meter	Glucose, Growth hormone & Cortisol
0	Check blood glucose using meter	Glucose, Growth hormone & Cortisol
15	Check blood glucose using meter	Glucose, Growth hormone & Cortisol
30	Check blood glucose using meter	Glucose, Growth hormone & Cortisol
60	Check blood glucose using meter	Glucose, Growth hormone & Cortisol
90	Check blood glucose using meter	Glucose, Growth hormone & Cortisol
120	Check blood glucose using meter	Glucose, Growth hormone & Cortisol

Samples

Growth Hormone 1.2 mL clotted blood (white top)

Cortisol 1.2 mL lithium heparin (orange top) or clotted blood (white top)

Glucose 1.2 mL venous blood in a fluoride oxalate tube (yellow top)

Record actual sample collection times on the printed barcodes.

Management of hypoglycaemia

- If symptomatic, give glucose (3 mL/kg of i.v. 10% glucose) - **INFORM DOCTOR**
- Give feed if able to tolerate, if not intravenous maintenance fluids, 10% glucose + sodium chloride (e.g., 10% glucose/0.45% sodium chloride)
- Recheck finger prick BG every 15 min until glucose >4.0mol/L
- If BG remains low, consider further bolus and increase glucose concentration/ fluid rate. Consider hydrocortisone bolus.
- CONTACT ENDOCRINE CONSULTANT ON CALL IF ANY CONCERNS

Interpretation

Interpretation is only possible if adequate hypoglycaemia (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 sufficient to stimulate a plasma GH concentration exceeding 7 µg/L.

Hypoglycaemia of this magnitude should also cause an increase in the plasma cortisol to concentrations exceeding 430 nmol/L.

References

1. Managed clinical network of Scottish Paediatric Endocrine Group (SPEG MCN) Dynamic function test handbook for Clinicians January 2012
2. 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 Test of Anterior Pituitary Function (1) - Arginine, TRH, GnRH, Synacthen

Test Name: Please request tests separately (CHILD THYROTROPIN STIMULATION DFT, CHILD SYNACTHEN DFT, CHILD GNRH STIMULATION TEST, CHILD ARGININE STIMULATION DFT)

Principle

Simultaneous administration of GH stimulants and hypothalamic releasing hormones GnRH and TRH does not alter the hormonal response from that seen during a specific single provocation test. 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 disease.

Precautions

- The GnRH test cannot be performed if the child has been primed with sex steroid to stimulate GH response.

Side Effects

- Arginine may cause nausea and some irritation at the infusion site, although this is limited by the infusion over a 15-minute time period.
- Arginine may also rarely cause anaphylaxis.
- In children with suspected hypopituitarism prolonged fasting may induce hypoglycaemia. Blood glucose should be checked by POCT in these patients whenever a sample is taken.
- TRH administration can give patients the desire to urinate. It is therefore advisable to ask older children to empty their bladder before commencing the test.
- Asthmatic patients should be carefully monitored throughout the test.
- Order the TRH (protirelin) from pharmacy at least 24 hours in advance.

Preparation

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

Protocol

1. Insert an indwelling 22-gauge, blue cannula. Take blood samples for growth hormone and U&E (basal t = -30). Cannulation may cause growth hormone to rise; therefore, the patient should rest for 30 min before the test is commenced.

2. Take blood samples for GH, cortisol, prolactin, TSH, fT4, LH, FSH, testosterone (boys) and oestradiol (girls) before commencing the infusion of arginine (t = 0). 4 x 2 mL samples are required.

3. Start arginine infusion. Immediately following the start of the arginine infusion (t = 0 min), check the patient's blood glucose level using a meter.

Generic	***Route***	***Dose***	***Frequency***
L-arginine monohydrochloride (10% solution in 0.9% sodium chloride)	*i.v*	*0.5g/kg body weight up to a maximum of 30g*	*Infusion over 15 mins*

4. Take blood samples for growth hormone 15, 30, 45, 60, 90 and 120 min after **the start** of the arginine infusion (i.e., 15 min sample should be taken during the arginine infusion). At each time point also check the blood glucose of the patient using a blood glucose meter.
5. TRH (protirelin), GnRH (Gonadorelin) and synacthen (Tetracosatide) are all given i.v. following the arginine infusion:

Generic	Route	Dose	Frequency
Protirelin (TRH)	*i.v (slowly over 2 minutes)*	*5 micrograms/kg (to a maximum of 200 micrograms)*	*Bolus*

Age	Generic	Route	Dose	Frequency
<1 year	Gonadorelin	*i.v*	*2.5 micrograms/kg*	*Bolus*
≥ 1 year	Gonadorelin	*i.v*	*100 micrograms*	*Bolus*

Age	Generic	Brand	Route	Dose	Frequency	Comments
<1 month	*Tetracosatide*	*Synacthen*	*i.v*	*36 micrograms/kg*	*Bolus*	
1-12 months	*Tetracosatide*	*Synacthen*	*i.v*	*125 micrograms*	*Bolus*	*Use 36 micrograms/kg for preterm babies who remain in hospital.*
>1 year	*Tetracosatide*	*Synacthen*	*i.v*	*250 micrograms*	*Bolus*	

N.B. For the combined TRH/GnRH/Synacthen omit the first part of the schedule relating to Arginine.

Time Points and samples:

		Arginine		TRH	GnRH	Synacthen	Extra Tests
Time (min) post infusions	**Blood sample**	GH	Blood glucose meter	TSH	LH/FSH	Cortisol	
-30	1.2 mL Clotted 1.2 mL LiHep	+	+				U&E
0	1.2 mL Clotted 1.2 mL LiHep	+	+	+	+	+	Prolactin, fT4, LH, FSH, Testosterone or Oestradiol
15	1.2 mL Clotted 1.2 mL LiHep	+	+				
20	1.2 mL Clotted 1.2 mL LiHep			+			
30	1.2 mL Clotted 1.2 mL LiHep	+	+		+	+	
45	1.2 mL Clotted 1.2 mL LiHep	+	+				
60	1.2 mL Clotted 1.2 mL LiHep	+	+	+	+		
90	1.2 mL Clotted 1.2 mL LiHep	+	+				
120	1.2 mL Clotted 1.2 mL LiHep	+	+				

Interpretation

As for individual stimulation tests.

References

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

Combined Test of Anterior Pituitary Function (2) - Glucagon, TRH, GnRH

Test Name: Please request tests separately (CHILD THYROTROPIN STIMULATION DFT, CHILD SYNACTHEN DFT, CHILD GNRH STIMULATION TEST, CHILD GLUCAGON STIMULATION FOR GROWTH HORMONE DFT)

Principle

Simultaneous administration of GH stimulants and hypothalamic releasing hormones GnRH and TRH does not alter the hormonal response from that seen during a specific single provocation test. 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 disease.

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 phaeochromocytoma 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 mobilise glycogen may result in hypoglycaemia.
- The test should not be carried out in patients with severe hypocortisolaemia (9.00am level <100 nmol/L)
- Thyroid function must be normal as thyroxine deficiency may reduce the GH and cortisol response.

Side Effects

- Glucagon can commonly result in nausea and abdominal pain (30%) and patients may rarely vomit.
- In children with suspected hypopituitarism prolonged fasting may induce hypoglycaemia. Blood glucose should be checked by POCT in these patients whenever a sample is taken.
- Asthmatic patients should be carefully monitored.
- TRH administration can give patients the desire to urinate. It is therefore advisable to ask older children to empty their bladder before commencing the test.
- Order the TRH (protirelin) from pharmacy at least 24 hours in advance.

Preparation

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

Protocol

1. Insert an indwelling 22-gauge, blue cannula and take a blood sample for growth hormone and U&E (t = -30). Cannulation may cause growth hormone to rise; therefore, the patient should rest for 30 min before the test is commenced.

2. Take blood samples for growth hormone, cortisol, prolactin, TSH, fT4, LH, FSH, testosterone (boys) or oestradiol (girls; BASAL, t = 0). Check the patient's blood glucose level using a meter.

3. ***Infusions and Injections***

Generic	***Route***	***Dose***	***Frequency***
Protirelin (TRH)	*i.v (slowly over 2 minutes)*	*5 micrograms/kg (to a maximum of 200 micrograms)*	*Bolus*

Generic	Route	Dose	Frequency
Glucagon	*i.m*	*30 micrograms/kg of body weight up to a maximum dose of 1 mg.*	*Bolus*

Gonadotrophin Releasing Hormone

Age	Generic	Route	Dose	Frequency
<1 year	Gonadorelin	*i.v*	*2.5 micrograms/kg*	*Bolus*
≥ 1 year	Gonadorelin	*i.v*	*100 micrograms*	*Bolus*

Time Points and samples:

		Glucagon		TRH	GnRH	Extra Tests
Time (min) post infusions	**Blood sample**	GH	Blood glucose meter	TSH	LH/FSH	
-30	1.2 mL Clotted 1.2 mL LiHep	+	+			**U&E**
0	1.2 mL Clotted 1.2 mL LiHep	+	+	+	+	**Prolactin, fT4, LH, FSH, Testosterone or Oestradiol**
20	1.2 mL Clotted 1.2 mL LiHep			+		
30	1.2 mL Clotted 1.2 mL LiHep				+	
60	1.2 mL Clotted 1.2 mL LiHep	+	+	+	+	
90	1.2 mL Clotted 1.2 mL LiHep	+	+			
120	1.2 mL Clotted 1.2 mL LiHep	+	+			
150	1.2 mL Clotted 1.2 mL LiHep	+	+			
180	1.2 mL Clotted 1.2 mL LiHep	+	+			

Samples
See table.

Interpretation
As for individual stimulation tests.

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

Glucose Suppression Test for Growth Hormone

Principle

Acromegaly in adults and gigantism in children are relatively rare diseases that are caused by persistent growth hormone (GH) hypersecretion. The estimated prevalence of acromegaly in adults is 60 cases per million with 3-4 new cases per million per year. In gigantism in children, the disease is diagnosed prior to epiphyseal fusion, leading to excessive tall stature. After completion of growth, the clinical symptoms become more similar to those in acromegalic adults like coarse facial features, acral changes, hyperhydrosis, headaches and visceromegaly. GH secretion is part of the counter-regulatory defence against hypoglycaemia and physiological GH secretion is inhibited by hyperglycaemia. In acromegaly, GH secretion is autonomous and does not suppress and may paradoxically rise with hyperglycaemia.

Indication

- This test is used to investigate clinical suspicion of acromegaly or gigantism. Baseline GH values cannot be used to exclude acromegaly since elevated GH may occur with stress and low values are seen in up to 8% of acromegalic patients who are subsequently identified by the failure of GH to suppress during a GTT.

Precautions

- This test is unnecessary in diabetics who have already shown GH suppression in the presence of hyperglycaemia.

Side Effects

- Some subjects feel nauseated and may have vaso-vagal symptoms during this test.

Preparation

- The diet over the preceding 3 days should contain adequate carbohydrate (approx. 60% of calories).
- The patient should be fasted overnight for 10 to 14 hours (water only allowed) and should rest throughout the test.
- Physical exercise is not allowed in the morning prior to and/or during the test.
- The test should be performed in the morning.

Protocol

- Prepare the glucose load as **ONE** of the following:
    - ***POLYCAL® (Nutricia Clinical) liquid.*** POLYCAL contains 0.66g anhydrous glucose per mL (or 1.51 mL contains 1g anhydrous glucose). The dose of POLYCAL must be adjusted for the weight of the child at a dose of 2.64 mL POLYCAL/kg body weight (to a maximum of 113 mL POLYCAL, equivalent to a 75g glucose load). Add water to make up to a volume of 200 mL.

      OR

    - ***Glucose tolerance test (Rapilose) Solution:*** Contains 75g anhydrous glucose in 300 mL. For children weighing less than 43kg, the dose is 7 mL (1.75g anhydrous glucose)/kg body weight. The total dose should not exceed 75g anhydrous glucose. If the volume is less than 200 mL, add water to make up to 200 mL.

- Insert a reliable cannula and take a basal blood sample for glucose, growth hormone, IGF1 and IGF-BP3 (t = 0).
- The child should drink the glucose load over a period of about 5 min.
- Take further blood samples for glucose and growth hormone 30, 60, 90 and 120 post administration of the glucose drink.

Time Points:

Time post glucose drink (min)	Procedure	Blood Sample
0	-	Growth hormone, glucose, IGF1 and IGF-BP3
30	-	Growth hormone, glucose
60	-	Growth hormone, glucose
90	-	Growth hormone, glucose
120	-	Growth hormone, glucose
150	-	Growth hormone, glucose

Samples

Growth Hormone 1.2 mL clotted blood (white top)

Glucose 1.2 mL fluoride oxalate tube (yellow top)

IGF1 + IGF-BP3 1.2 mL clotted blood (white top)

Interpretation

- Normal subjects are likely to exhibit suppression of GH to < 0.5 µg/L during the test, but the results should be interpreted in conjunction with IGF-1 results.
- High basal levels which fail to suppress, sometimes with a paradoxical rise in GH levels is characteristic of GH hypersecretion.
- A paradoxical rise in GH may occur during the OGTT during normal adolescence.
- GH may fail to suppress due to chronic renal failure, liver failure, active hepatitis, anorexia nervosa, malnutrition, hyperthyroidism, diabetes and adolescence.
- Basal IGF-BP3 levels may be a useful adjunct. Patients with untreated acromegaly consistently have significantly raised random serum IGF-1 and IGFBP-3 levels, showing no overlap with normal individuals.

References

1. Freda P.U. (2009) Monitoring of acromegaly: what should be performed when GH and IGF-1 levels are discrepant? *Clin Endocinol* **71**: 166 – 170

IGF-1 Generation Test

Test Name: Please request tests separately.

Principle

Growth hormone is administered to the patient where there is strong suspicion of growth hormone 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 growth hormone 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[1].

Indication

- Diagnosis of growth hormone insensitivity

Precautions

- None reported.

Side Effects

- No significant effect of short-term Growth hormone use

Preparation

- None required.

Protocol

1. **Day 1** – take blood sample for IGF-1 estimation.
2. ***Administer growth hormone on days 1, 2, 3 & 4:***

Generic	*Brand (if applicable)*	*Route*	*Dose*	*Frequency*	*Comments*
Somatropin	*Genotropin Miniquick or similar if unavailable*	*Subcutaneously*	*0.1 units/kg body weight/day (i.e. 33 micrograms/kg body weight/day)*	*Daily for 4 days (day 1-day 4)*	

3. **Day 5** – take blood sample for IGF-1 estimation.

Samples

IGF-1 1.2 mL clotted blood (white top)

Interpretation

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

References

1. 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

Water Deprivation Test

Test Name: CHILD WATER DEPRIVATION TEST

This test is potentially dangerous and must be undertaken with great care. Patients unable to conserve water may rapidly become severely hypertonic during this test.

Arrangements for carrying out a Water deprivation Test:

When a decision is taken, either in clinic or on the ward, to perform a water deprivation test, to arrange for this test, please action as follows:

1. Inform Paediatric Endocrine Secretary.

2. Secretary to discuss with dates for admission with ETC.

    - Admission will be on Short Stay Ward from approx. 4.00 pm for overnight stay for bloods/osmolality etc. as per protocol, followed by early morning admission on MIU early the following morning for Water Deprivation Test.

3. Secretary contacts Duty Biochemist on extension 12255 to check if date suitable.

4. Duty Biochemist adds test to lab diary (for day after admission)

5. Secretary confirms date with ETC.

Principle

Water restriction in normal individuals results in the secretion of arginine vasopressin (AVP) from the posterior pituitary in order to reabsorb water from the distal renal tubules and concentrate urine. Failure of this mechanism results in a rise in plasma osmolality, due to water loss, and a dilute urine of low osmolality. The concentrating mechanism for urine is maintained in compulsive water drinking (CWD). AVP-deficiency (formerly known as cranial diabetes insipidus) is caused by a failure of AVP secretion whilst AVP-resistance (formerly known as nephrogenic diabetes insipidus) is caused by insensitivity of the renal tubules to AVP. The two AVP disorders can be distinguished by the administration of desmopressin (synthetic AVP).

Indication

- This test is used to distinguish AVP disorders from primary polydipsia and to identify whether AVP deficiency or resistance is present.
- A subjective thirst score may be performed at the same time and requires copies of the unit-less 100 mm linear visual analogue scale.

Precautions

- This test should not be performed if there is evidence of the kidney's ability to concentrate urine e.g. spot urine osmolality >750 mmol/kg.
- Other causes of polyuria and polydipsia ***MUST*** be excluded before proceeding with the test. These include:
    - Diabetes mellitus
    - Hypoadrenalism
    - Hypercalcaemia
    - Hypokalaemia
    - Hypothyroidism
    - Urinary infections
    - Chronic kidney disease
    - Therapy with carbamazepine, chlorpropamide or lithium

Cortisol insufficiency must be treated prior to doing a water deprivation test as it interferes with the ability to excrete water and can mask an AVP disorder.

Side Effects

- Patients with AVP deficiency/ resistance may become severely water depleted during this test and ***MUST*** be carefully monitored (by weighing the patient and quantifying urine output regularly) throughout the test.

Preparation

The laboratory MUST be notified AT LEAST 24 hrs before the test, ideally with more notice. Please see instructions on previous page. Osmolality results are required as soon as possible after the specimens have been collected.

- Before considering the test, polyuria must be established with an accurate 24 hr urine output measurement. Urine output >4 mL/kg/hr in infants and children >1 year old is suggestive of polyuria.
- *The overnight test is reserved for situations where the diagnosis cannot be easily made by stopping oral fluid intake for a few hours and obtaining sodium and osmolality measurements.*
- *Children with massive polyuria (>4L/24 hr) should start the test in the morning when medical staff are present as the test will usually last 2–4 hrs.*
- Thyroid and adrenal function must be normal or adequately replaced.
- The patient must be kept under close surveillance throughout the test to avoid surreptitious water drinking and in order to be monitored for any signs of dehydration.
- During the test the child should be allowed to eat snacks with no fluid intake of milk, juice or water. Dry snacks such as biscuits or crisps would be preferable.

Protocol

1. The night before the test (at 2200h), take blood for bedside glucose, plasma osmolality, urea, electrolytes, glucose and copeptin.
   - *The test can only be carried out if the plasma osmolality is <295 mmol/kg.*
   - *Plasma osmolality can be calculated from the urea, electrolyte and glucose results using the formula:*

     Calculated plasma osmolality = (2 x Na) + Glucose + Urea
   - *The osmolality sample will be analysed by the lab first thing in the morning before the test commences.*

2. If the test is to proceed, weigh the patient undressed, record the weight and insert a reliable i.v. cannula.

3. Assess the patient:
   - If there is a low level of suspicion of an AVP disorder and the patient is >2 years of age, stop all fluid intake at midnight.
   - If there is a high index of suspicion of an AVP disorder (i.e., patients are polyuric or borderline hyperosmolar), or if the child is <2 years of age, fluid restriction should commence in the morning.
4. **Print out the water deprivation template on page 49 and fill in.**

5. At 0900h weigh the patient undressed and record the weight. Calculate and record 5% of the weight. Collect blood and urine samples for bedside glucose, osmolality, urea, electrolytes and copeptin. The samples should be sent **immediately** to the Biochemistry laboratory.
   If the osmolality is >295 mmol/kg the water deprivation test must not be undertaken.

6. On hourly basis, undertake the following and record on the table below:
   a. **Record fluid input and output – this must be strictly charted.**

b. **Collect blood for bedside glucose, plasma urea and electrolytes, plasma osmolality and plasma copeptin.**
c. **Collect urine sample for urine osmolality.**
d. **Measure and record heart rate and blood pressure**
e. **Weigh the child and record on table. For measurement of weight, the child should be undressed or measured wearing the same clothing. Inform paediatric endocrine team if weight loss of more than 5% occurs. They will consider termination of test with administration of desmopressin.**

7. The test is normally continued until 3 consecutive urines have shown a total rise in urine osmolality of <30 mmol/kg (normally about 12 midday) or until either:

- The urine osmolality exceeds 750 mmol/kg (or 500 mmol/kg in infants)
- 5% of initial weight is lost or thirst is unbearable.
- Plasma osmolality exceeds 300 mmol/kg.

N.B. It may be necessary to prolong the test in compulsive water drinking, especially if the child has been drinking excessively immediately prior to the start.

7. At 12 midday, or when the test is terminated, take blood samples for urea, electrolytes, osmolality and copeptin, along with a urine sample for osmolality.
N.B. If 5 % weight loss or extreme distress occurs, give desmopressin (5 micrograms intra-nasally or 0.3 microgram i.m.) and free fluids immediately after test is terminated.

8. If the child shows no evidence of urinary concentration, proceed with the desmopressin test to allow differentiation between AVP-deficiency and AVP-resistance.

9. It is unlikely that child has an AVP disorder if the child fails to pass urine during the duration of water deprivation test and clinically remains well.

Desmopressin Test

Test Name: CHILD WATER DEPRIVATION TEST DDAVP EXTENSION

1. Allow the patient to drink ***but not excessively*** or a dilutional hyponatraemia may occur.
N.B. Fluid intake should be no more than twice the volume of urine passed during fluid restriction. Fluid intake should be monitored closely.

2. Give subcutaneous **or** intranasal desmopressin as follows ***(Desmopressin treatment must be discussed with the on-call endocrinologist):***

Age	*Generic*	*Route*	*Dose*	*Frequency*
0-2 years	*Desmopressin*	*Subcutaneous*	*0.04 micrograms/kg maximum initial dose 0.4 micrograms*	*Bolus*
2-12 years	*Desmopressin*	*Subcutaneous*	*0.4 - 1 microgram*	*Bolus*
2-12 years	*Desmopressin*	*Intranasal*	*10 – 20 micrograms*	*Bolus*
>12 years	*Desmopressin*	*Subcutaneous*	*0.7 - 1 microgram*	*Bolus*
12-18 years	*Desmopressin*	*Intra-nasal*	*20 micrograms*	*Bolus*

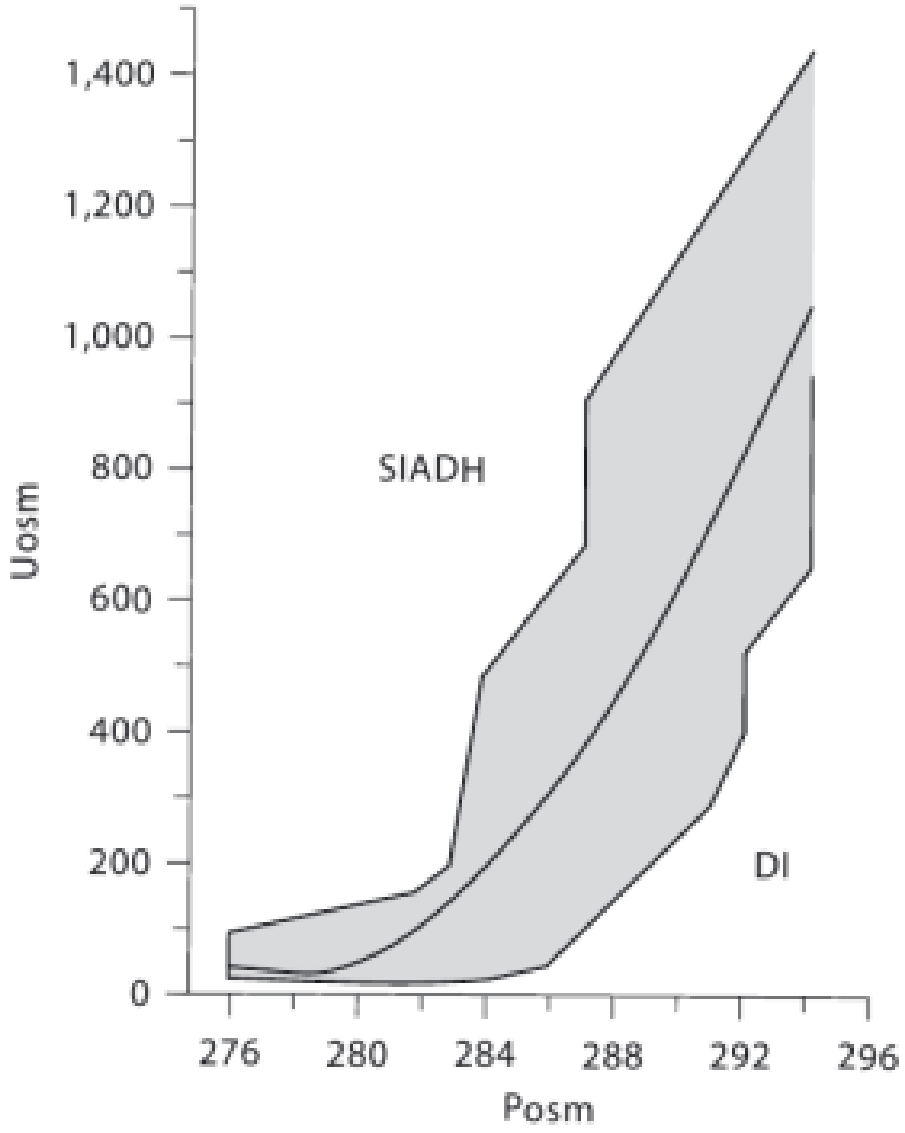
3. Collect blood and urine samples for osmolality hourly (if possible) for the next 4 hours. If necessary, this can continue to 6 hours to obtain a diagnostic result allowing hourly blood glucose monitoring and allowing the child to eat dry food. Stop the test if the urine osmolality reaches >750 mmol/kg.

Samples

Na, K, Urea & Plasma Osmolality	1.2 mL lithium heparin blood (orange top)
Glucose	1.2 mL fluoride oxalate tube (yellow top)
Urine osmolality	1-2 mL urine in a plain bottle
Copeptin	1.2 ml lithium heparin (orange top). The laboratory will only send copeptin for analysis if the urine and plasma osmolality results are indicative of an AVP disorder. The sample collected at the time of the highest osmolality will be sent.

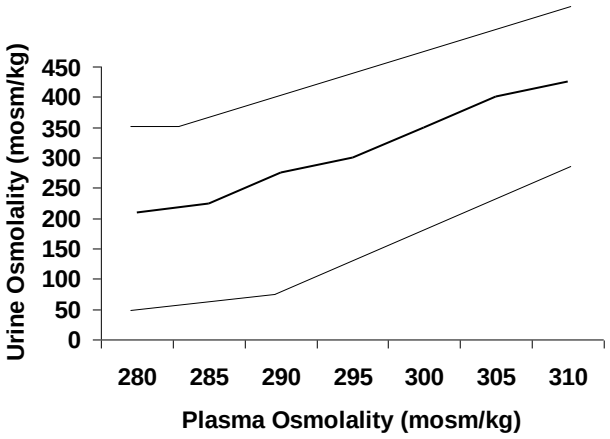
Interpretation

Normal and CWD:	Plasma osmolality does not exceed 295 mmol/kg and the urine osmolality rises three-fold to >750 mmol/kg.
AVP-deficiency:	Plasma osmolality >295 mmol/kg with inappropriately dilute urine (<300 mmol/kg). Desmopressin produces normally concentrated urine.
AVP-resistance:	As for Central DI, but desmopressin produces no response.
Partial AVP disorder:	Patients have moderate elevation of plasma osmolality and urine osmolality typically between 300-750 mmol/kg.
Copeptin:	There are currently no reference ranges for copeptin in children. The following ranges are derived from limited studies in adult populations: <u>Baseline copeptin levels (without prior thirsting):</u> ≥21.4 pmol/L – Suggests AVP-resistance. <21.4 pmol/L – Suggests other polyuria-polydipsia syndromes (including AVP-deficiency) >5 pmol/L – Suggests that AVP-deficiency is unlikely (even with a normal serum sodium/osmolality) <2.6 pmol/L – Suggests AVP-deficiency. <u>Stimulated copeptin levels (plasma osmolality >300 mmol/kg):</u> <4.9 pmol/L – Suggests AVP-deficiency >6.5 pmol/L - Suggests primary polydipsia

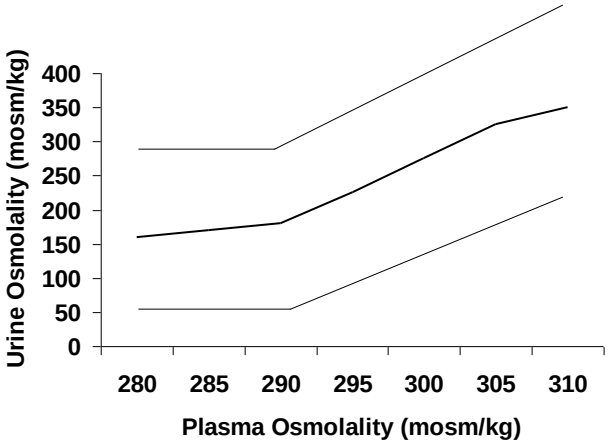


Plasma-urine osmolality relationship in adults and children adapted from Harrison's principles of internal medicine 1998[2].

PLASMA-URINE OSMOLALITY RELATIONSHIP IN FULLTERM NEWBORNS



PLASMA-URINE OSMOLALITY RELATIONSHIP IN PRETERM INFANTS



Plasma urine osmolality relationships in full term and pre-term infant graphs are taken from Great Ormond street protocol book.

Water deprivation test template

Date:_____

Name_____ Date of Birth:_____ Hospital Number:_____

The night before the test (at 2200h), take blood for bedside glucose, plasma osmolality, urea, electrolytes, glucose and copeptin.

Fluids restricted since (if overnight fluid restricted):

Water deprivation test started at (ideally 9 am):

Patient weight	Kg	Calculate weight minus 5 %	Kg

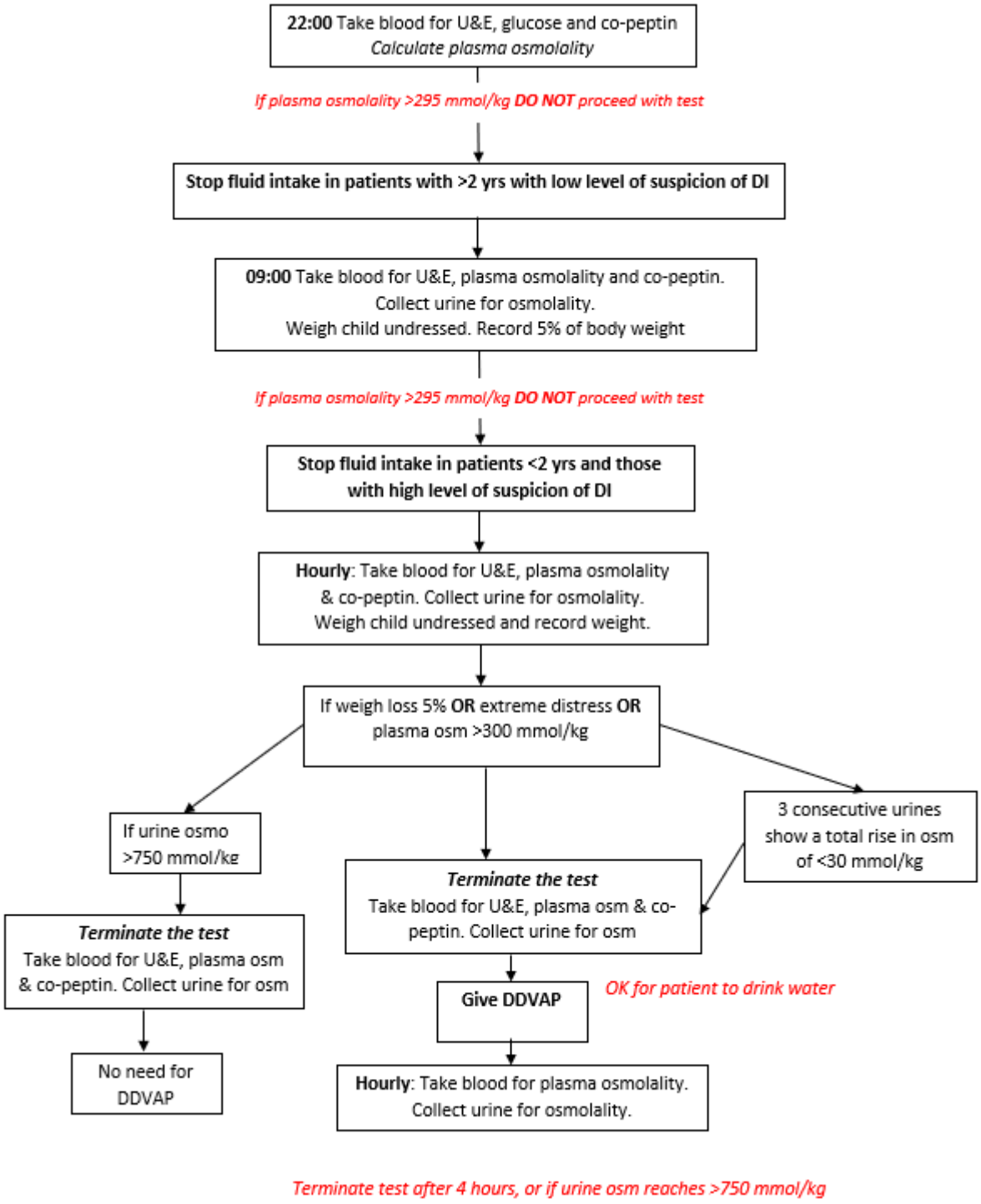
Test must be stopped if weight loss exceeds 5 %.
Test must be stopped if serum osmolality exceeds 300 mOsm/kg

Date/Time	Weight	Bedside Blood Glucose	HR	BP	Serum osmolality	Urine Osmolality	Urine output

Please note - the test should only be stopped after discussion with the named consultant.

The test is normally continued until:

- 3 consecutive urines have shown a total rise in urine osmolality of <30 mmol/kg (normally about 12 midday
- The urine osmolality exceeds 750 mmol/kg (or 500 mmol/kg in infants)
- 5% of initial weight is lost or thirst is unbearable.
- Plasma osmolality exceeds 300 mmol/kg.



References

1. Di Iorgi N., Napoli F., Allegri A.E.M., Olivieri I., Bertelli E., Gallizia A., Rossi A. & Maghnie M. (2012) Diabetes Insipidus – Diagnosis and management. *Horm Res Paediatr* **77**: 69 – 84
2. Feingold KR, Anawalt B, Boyce A, et al., editors. Endotext. South Dartmouth (MA): MDText.com, Inc.; 2000
3. Harrison's principles of internal medicine 14th Edition 1998.

Hypertonic Saline Infusion Test

Test Name: CHILD HYPERTONIC SALINE INFUSION TEST

Not commonly used

This test is potentially dangerous (it is very rarely used in children) and must be undertaken with great care. Patients unable to conserve water may rapidly become severely hypertonic during this test.
This test requires a doctor to be present throughout.

Principle

This test is designed to stress the integrity of the renal-AVP axis, in order to assess posterior pituitary function, providing reliable information regarding the relation between plasma osmolality and AVP. The infusion of hypertonic saline raises plasma osmolality and ensures maximal stimulation of AVP secretion. The failure of maximal renal concentration of urine does not help differentiate which organ is performing sub-optimally. The diagnosis can be seen by comparing the response of plasma AVP to plasma osmolality using the Newcastle chart (Prof P.H. Baylis).

Indication

- This test is performed if the results of the water deprivation test are equivocal: the test can be useful in differentiating partial forms of AVP disorders and to demonstrate normal osmoregulation in patients with primary polydipsia. This test is also indicated when investigating patients with adipsic or hypodipsic hypernatraemia. A subjective thirst score maybe performed at the same time and requires copies of the unit-less 100 mm linear visual analogue scale.

Precautions

- Contraindicated in patients with epilepsy, cerebral or cardiovascular disease.

Side Effects

- There is a serious risk of dehydration in patients with AVP disorders.
- The hypertonic saline may induce thrombophlebitis at the site of infusion.

Preparation

The laboratory MUST be notified AT LEAST 24 hrs before the test, ideally with more notice. Osmolality results are required as soon as possible after the specimens have been collected.

- Overnight food fast from midnight the day before the test
- Only water maybe drunk until the time of the test; other drinks are not permitted after midnight.
- No smoking during period of food fast
- Absolute food/fluid fast during the infusion period
- Assess to exclude the presence of any confounding factor e.g., hypercalcaemia, hypokalaemia, glycosuria or any other cause of a dilute solute diuresis, prior to commencing the test.
- Cortisol insufficiency must be treated prior to doing a water deprivation test as it interferes with the ability to excrete water and can mask AVP disorders.

Protocol

1. Patient instructed to empty bladder. Measure urine volume and osmolality
2. Weigh patient
3. Patient to lie supine where they will remain for the remainder of the test.
4. Insert cannula into antecubital veins of both arms. Allow patient to rest for 30 min.

5. Blood pressure monitored every 5 min during the 30 min preceding the test and throughout the infusion period.
6. Take blood for copeptin and osmolality
7. Repeat blood sample after 15 min
8. Begin infusion of 5% (0.85 mol/L) sodium chloride at 0.05 mL/kg/min for 2 hours into non-blood sampling arm via an indwelling cannula for a max of 3 hrs or until a plasma osmolality of 300 mmol/kg is achieved.
9. Take blood samples at 30 min intervals for copeptin and osmolality
10. Measure volume and osmolality on all urine passed.

Note time at which thirst is noted - if patient very thirsty during test, give ice chips

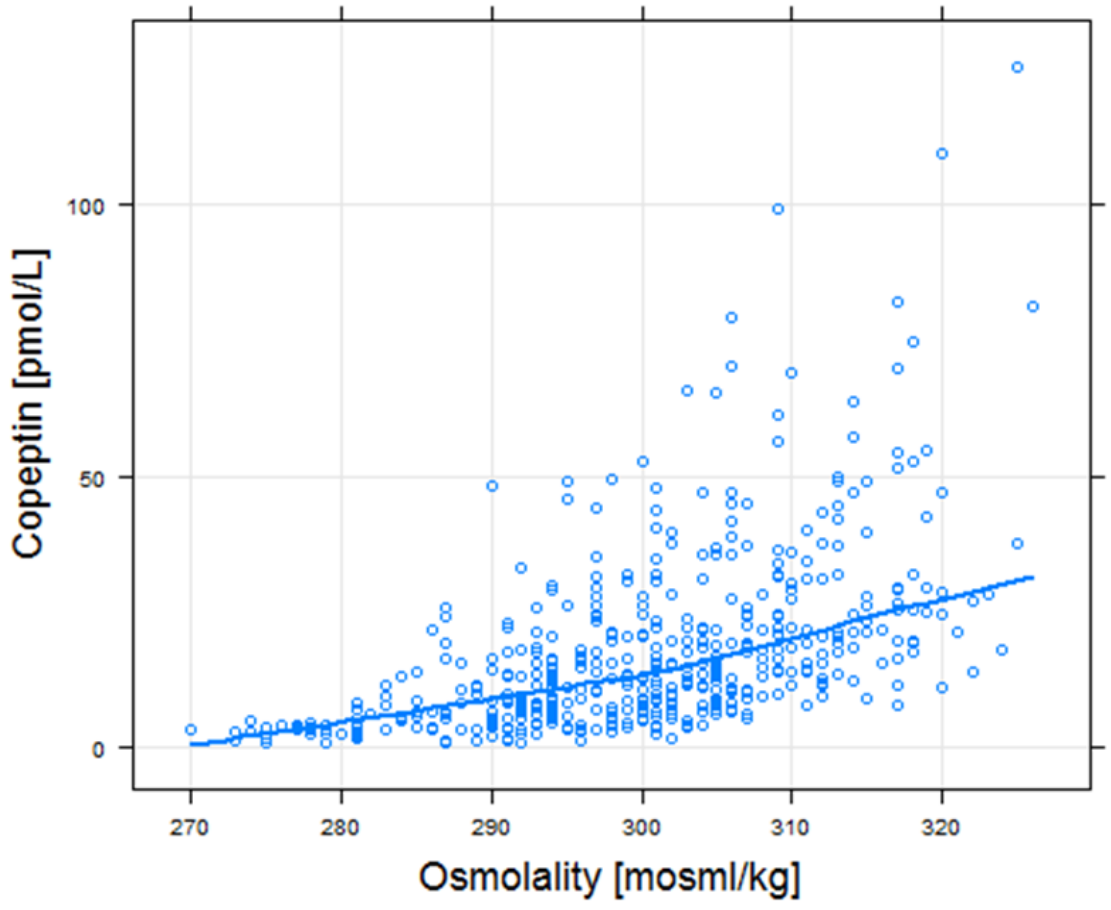
11. Take final blood sample 15 min after completion of infusion.
12. Record blood pressure, urine volume, blood sampling, patients' comments
13. Allow patient to drink after test. Avoid ingestion of large fluid volumes.

Samples

Plasma osmolality & sodium	1.2 mL lithium heparin blood (orange top)
Copeptin	1.2 mL lithium heparin blood (orange top)

Interpretation

Patients with primary polydipsia or AVP-resistance have normal AVP and copeptin release in response to the hyperosmolar state induced by this procedure. Patients AVP-deficiency have little or no rise in AVP and copeptin.



A. Correlation of Copeptin levels with plasma osmolality results from 91 healthy individuals (blue data points)[5]

Copeptin

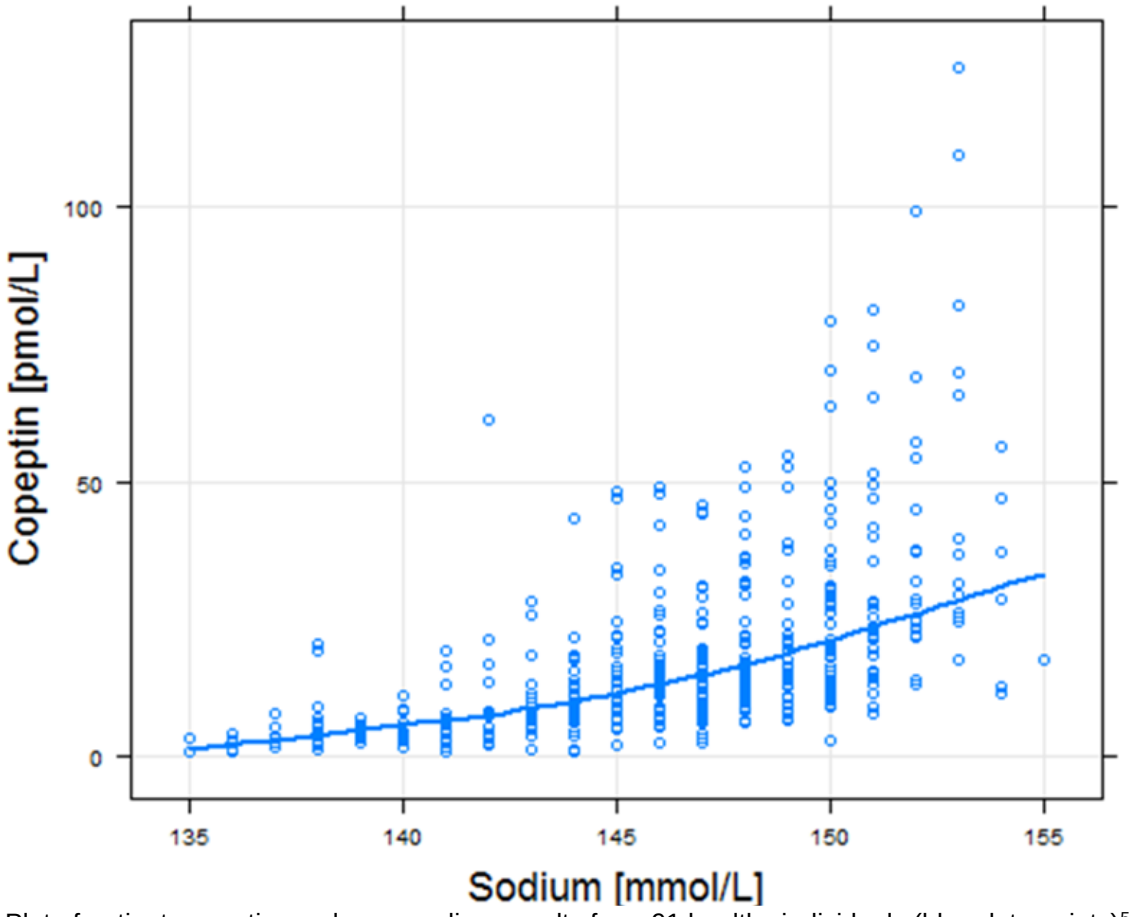
There are currently no reference ranges for copeptin in children.
The following ranges are derived from limited studies in adult populations:

Baseline plasma copeptin <2.6 pmol/L prior to hypertonic saline infusion - suggestive of complete AVP-deficiency.

Baseline plasma copeptin ≥21.4 pmol/L prior to hypertonic saline infusion suggestive of (partial or complete) AVP-resistance.

Baseline plasma copeptin >5 pmol/L – suggestive that AVP-deficiency is unlikely (even with a normal serum sodium/osmolality).
Plasma copeptin level of ≤4.9 pmol/L after hypertonic saline infusion - suggestive of partial or complete AVP deficiency.

Plasma copeptin level of >4.9 pmol/L after hypertonic saline infusion - suggestive of primary polydipsia. A higher cut-off value of 6.5 pmol/L distinguishes AVP-deficiency from primary polydipsia with increased accuracy based on newer data, with a value of >6.5 pmol/L being consistent with primary polydipsia[3].



Plot of patient copeptin vs plasma sodium results from 91 healthy individuals (blue data points)[5].

References

1. Baylis P.H. & Robertson G.L. (1980) Plasma vasopressin response to hypertonic saline infusion to assess posterior pituitary function. *J Roy Soc Med* **73**:255-60.
2. Baylis P.H. & Cheetham T. (1998) Diabetes insipidus. *Arch Dis Child* **79**: 84 – 89
3. Szinnai G et al. (2007) Changes in Plasma Copeptin, the C-Terminal Portion of Arginine Vasopressin during Water Deprivation and Excess in Healthy Subjects.
4. Fenske et al. (2018) A copeptin-based approach in the diagnosis of diabetes insipidus. New England Journal of Medicine. 2018;379(5):428–439
5. Schnyder et al.(2015) Physiological area of normality of copeptin in normal-to-hyperosmolar states. Endocrine Abstracts 37:EP706.

TRH Test

Test Name: CHILD THYROTROPIN STIMULATION DFT

Principle
TRH is used to stimulate the pituitary gland in order to assess the hypothalamic-pituitary-thyroid axis.

Indication
- The TRH test is used in the investigation of secondary hypothyroidism and allows for differential diagnosis of pituitary and hypothalamic causes of TSH deficiency.

Precautions
- Patients should be off thyroxine for 3 weeks prior to test, so it is rarely used in children on thyroxine.

Side Effects
- There are a number of reports in the literature of apoplexy post TRH in patients with pituitary adenoma. This is very rare, but patients should be counselled to contact the endocrine team if headaches or illness occur post-test.
- The test may cause mild flushing, nausea, headaches, abdominal and chest discomfort and a desire to micturate. Symptoms are usually seen at the time of injection.

Preparation
- Patient does not need to be fasted (unless combined with a test of GH secretion).
- Asthmatic patients should be carefully monitored throughout the test.
- As TRH may cause patients a desire to micturate, older children should be asked to empty their bladder before the test commences.
- Order the TRH (protirelin) from pharmacy at least 24 hours in advance.
- This test can be done in conjunction with other pituitary function testing. See ***Combined test of anterior pituitary function.***

Protocol
1. Insert an indwelling cannula and take baseline bloods for TSH and free T4 (t = 0).
2. ***Inject protirelin (TRH) slowly i.v. over 2 minutes.*** This should be completed whilst the patient is supine as side effects are most likely to occur during this period of time.

Generic	***Route***	***Dose***	***Frequency***
Protirelin (TRH)	*i.v*	*5 micrograms/kg (to a maximum of 200 micrograms)*	*Bolus*

3. Take further blood samples for TSH and fT4 20 and 60 min following the administration of TRH.

Time Points:

Time post TRH infusion (min)	**Blood Sample**
0	TSH, fT4
20	TSH
60	TSH

Samples
TSH and fT4 1.2 mL lithium heparin blood (orange top)

Interpretation

- A normal response is a TSH peak of 10 – 30 mU/L at 20 min, which will decrease by 60 min.
- An exaggerated response is often seen if basal TSH is elevated (BUT the test should only be used to investigate secondary hypothyroidism).
- In pituitary disease, TSH response is poor.
- A hypothalamic response is indicated by a peak at 20 min which remains elevated at 60 min.
- In both pituitary and hypothalamic types of TRH response, a low fT4 value may indicate need for replacement.

References

1. Ergür A.T., Evliyaoğlu O., Şiklar Z., Bilir P., Öcal G. & Berberoğlu M. (2011) Evaluation of thyroid functions with respect to iodine status and TRH test in chronic autoimmune thyroiditis. *J Clin Res Ped Endo* **3**(1): 18 – 21

Gonadotrophin-releasing hormone (GnRH) test

Test name: CHILD GNRH STIMULATION DFT

Principle
Gonadotrophin-releasing hormone (GnRH), secreted by the hypothalamus, stimulates the release of luteinising hormone (LH) and follicle-stimulating hormone (FSH) from the anterior pituitary gland.

Indication
- Investigation of pubertal disorders: precocious puberty and delayed puberty.
- Investigation of hypogonadotrophic hypogonadism suspected pre-pubertally.
- Monitoring of children with precocious puberty treated with GnRH analogues.

Precautions
- Avoid human chorionic gonadotrophin injections prior to the test and do not perform following priming for an arginine test.

Side Effects
- GnRH may rarely cause nausea, headache and abdominal pain.

Preparation
The patient need not be fasted (unless combined with a test of GH secretion).

Protocol
1. Insert a reliable cannula. Take blood for LH, FSH, testosterone or oestradiol (t = 0).

Administer GnRH:

Age	*Generic*	*Route*	*Dose*	*Frequency*
<1 year	Gonadorelin	*i.v*	*2.5 micrograms/kg*	*Bolus*
≥ 1 year	Gonadorelin	*i.v*	*100 micrograms*	*Bolus*

3. Take blood at + 30 min
+ 60 min

after the GnRH bolus for LH & FSH only

Samples
LH & FSH 1.2 mL lithium heparin (orange top)

Testosterone or Oestradiol 1.2 mL clotted blood (white top)

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

Prepubertal
Basal LH usually <1.0 IU/L. LH peak post-GnRH <6.0 IU/L. FSH peak greater than LH peak.

Peripubertal
Higher increments, especially if LH dominant, provide evidence of a pubertal pattern of gonadotrophin response. LH peak≥6.0 IU/L, with LH peak greater than FSH peak.

See table below for the reference ranges from Resende *et al*. 2007, for serum LH and FSH concentrations (AutoDELFIA assays) in normal subjects at different pubertal stages (n=316 for basal levels, n=106 for GnRH stimulated levels).

Pubertal Delay and Pubertal failure
In children with suspected hypogonadotrophic hypogonadism, a complete lack of response supports the diagnosis. A measurable but low response has limited predictive value (may also occur in constitutional delay of puberty). In primary gonadal failure, the basal LH and FSH are elevated and the response to GnRH is exaggerated. High basal FSH levels in the presence of low oestradiol levels may suggest ovarian failure.

Premature thelarche and thelarche variant
There may be a FSH predominant response, with LH usually in the pre-pubertal range.

Precocious puberty
In gonadotrophin-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 gonadotrophin-dependent precocious puberty basal LH and FSH levels are usually elevated and the response to GnRH is exaggerated. A LH dominant rise is usually observed, with LH levels usually >7.0 IU/L and more commonly >10.0 IU/L in established puberty.

Precocious puberty (treated)
Suppressed basal LH and FSH and flat response to GnRH indicate adequate treatment with GnRH analogues.

Table - Concentration of serum LH and FSH (AutoDELFIA assays), expressed as mean and 5th and 95th percentiles, in normal subjects at different pubertal stages (n=316 for basal levels, n=106 for GnRH stimulated levels)

Pubertal Stage	Males				Females			
	Basal		GnRH-stimulated peak		Basal		GnRH-stimulated peak	
	LH (IU/L)	FSH (IU/L)	LH (IU/L)	FSH (IU/L)	LH (IU/L)	FSH (IU/L)	LH (IU/L)	FSH (IU/L)
$T1_1$ (<2.6 yr)	<0.6	1.0 (1.0-1.4)	N/A	N/A	<0.6	3.7 (1.0-8.3)	N/A	N/A
$T1_2$	<0.6	1.1 (1.1-1.6)	2.2 (1.1-3.3)	5.7 (2.4-10.6)	<0.6	1.6 (1.0-3.4)	2.1 (0.6-4.2)	11.7 (1.9-27.1)
TII	1.3 (0.6-2.7)	1.8 (1.0-4.3)	15.6 (1.9-31.0)	3.6 (1.4-10.2)	1.0 (0.6-N/A)	2.3 (1.0-4.8)	5.3 (0.6-12.5)	6.5 (1.8-13.2)
TIII	1.4 (0.6-2.5)	2.1 (1.0-5.5)	16.1 (7.3-32.0)	4.2 (1.1-13.0)	2.9 (0.6-5.0)	3.9 (2.6-5.1)	21.0 (14.6-31.0)	7.9 (5.9-12.0)
TIV	1.6 (0.7-2.5)	2.1 (1.0-5.2)	17.3 (12.0-28.0)	4.8 (1.7-12.0)	3.1 (1.0-6.0)	4.0 (1.5-7.2)	26.2 (10.4-54.5)	8.6 (4.0-18.0)
TV	4.7 (2.4-8.2)	3.2 (1.2-5.7)	28.9 (9.5-56.3)	5.3 (1.8-12.0)	5.7 (0.6-15.4)	4.1 (1.0-7.3)	37.9 (9.7-114.0)	9.2 (2.8-18.8)

References

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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

3-day HCG Stimulation Test

Test Name**:** CHILD 3 DAY HCG STIMULATION DFT

Principle

Human chorionic gonadotrophin (hCG) is a polypeptide hormone and shares a common subunit with LH. It stimulates testicular Leydig cells to secrete androgens via the LH receptors. Children aged 6 months to 8 years frequently have undetectable basal gonadal steroids in plasma and gonadal function can only be assessed by Leydig cell stimulation using hCG.

Indication

- To detect functioning testicular tissue in the investigation of male hypogonadism, ambiguous genitalia, micropenis, delayed puberty and/or undescended testes. The test should be performed even if the gonads are impalpable (and the karyotype is XY or XY mosaic).
- To define enzyme blocks in testosterone biosynthesis.
- A urine steroid profile (after 1-3 months of age) may be more helpful for investigation of possible 5-alpha reductase deficiency, as blood results can be misleading.
- For investigation of possible 17-β-hydroxy-steroid dehydrogenase deficiency, a hCG stimulation test is more reliable than a urine steroid profile.

Precautions

- In boys with normal testes there may be some virilisation (increase in testicular size, erections).
- The test should not be performed before 2 weeks of age.
- If a GnRH test is planned, this should be carried out before the HCG test (or > 6 weeks after) as HCG has a long half-life.

Side Effects

- Headaches and/or tiredness are reported side effects.

Preparation

- None required.

Protocol

3 Day Protocol:

1. **Day 1** - Between 8.00 a.m. and 9.00 a.m. collect baseline blood samples for testosterone, androstenedione and dihydrotestosterone

2. Immediately following collection of baseline blood samples, ***give human chorionic gonadotrophin as follows:***

Generic	***Route***	***Dose***	***Frequency***
human chorionic gonadotrophin	*i.m*	*500 units if weight < 5kg* *1000 units if weight 5 - 10kg* *1500 units if weight 10 - 15kg* *3000 units if weight above 15kg*	*Bolus*

3. **Day 4** - Repeat blood sample 72 hours after hCG injection for testosterone, dihydrotestosterone and androstenedione.

If the results of the 3-day test are equivocal then consider performing the 3-week hCG stimulation test.

Samples

Testosterone, DHT & Androstenedione 1.2 mL clotted blood (white top)

Urinary Steroid Profiling 24hr timed urine in a plain bottle if indicated (collection after injection usually preferred e.g., 24hr prior to day 4

visit, check with consultant). Random/ 4-hour timed sample less reliable but is acceptable. Collection on day 4 usually preferable to day 1.

Interpretation

The normal testosterone response depends on the age of the patient. In infancy, a normal testosterone increment after hCG may vary from 2-fold to 10- or even 20-fold. During childhood, the increment is between 2- and 9-fold. During puberty, as the basal concentration is higher, the increment is less, i.e. 2- to 3-fold. In the absence of testes, no response to testosterone occurs.

An absent response with an exaggerated LH/FSH response to LHRH stimulation indicates primary gonadal failure or anorchia. If there is a defect in testosterone biosynthesis, there will be an increase in precursor steroid secretion following HCG stimulation.

There are reported errors in the interpretation of the hCG stimulation test in boys ~8yrs of age with increased Testosterone: DHT in the 5α-reductase range.

Samples are sent to Leeds General Infirmary for analysis.
Interpretation (from Leeds):

	Testosterone (nmol/L)	DHT (nmol/L)	Testosterone /DHT ratio post hCG*	Androstenedione (nmol/L)	Androstenedione/ Testosterone ratio post HCG
Normal male adults	8 - 27	0.4-1.9	< 15	1.3 -5.8	<1.0
Normal children (6 months – puberty)	< 0.9	< 0.25	< 15	<1.4	<1.0
5α-reductase deficiency (6 months – puberty)	<0.5	N/A	> 20	N/A	N/A
17-β-hydroxy-steroid dehydrogenase deficiency	N/A	N/A	N/A	N/A	Raised >2.0**

* Testosterone /DHT ratio post hCG 15-20: 5-alpha-reductase deficiency cannot be excluded

** Androstenedione/ Testosterone ratio post HCG: Males, all ages: <1.0 – Likely excludes 17β-hydroxysteroid dehydrogenase deficiency. Adults: >3.0 – Indicative of 17β-hydroxysteroid dehydrogenase deficiency.

References

1. Maimoun L., Philibert P., Cammas B., Audran F., Bouchard P., Fenichel P., Cartigny M., Pienkowski C., Polak M., Skordis N., Mazen I., Ocal G., Berberoglu M., Reynaud R., Baumann C., Cabrol S., Simon D., Kayemba-Kay's K., De Kerdanet M., Kurtz F., Leheup B., Heinrichs C., Tenoutasse S., Van Viet G., Gruters A., Eunice M., Ammini A.C., Hafez M., Hochberg Z., Einaudi S., Mawlawi H.A., del Valle Nunez C.J., Servant N., Lumbroso S., Paris F. & Sultan C. (2011) 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
3. Leeds Children's Hospital Paediatric Endocrinology Dynamic Function Tests: Valid Jan 2022 to Jan 2025. hCG Stimulation Test

3-week HCG Stimulation Test

Test Name: CHILD 3 WEEK STIMULATION TEST DFT

Principle

Human chorionic gonadotrophin (hCG) is a polypeptide hormone and shares a common subunit with LH. It stimulates testicular Leydig cells to secrete androgens via the LH receptors. Children aged 6 months to 8 years frequently have undetectable basal gonadal steroids in plasma and gonadal function can only be assessed by Leydig cell stimulation using hCG.

Indication

- In the event of an equivocal result from the 3-day HCG stimulation test, the 3-week HCG stimulation test should be used.

Precautions

- In boys with normal testes there may be some virilisation (increase in testicular size, erections).
- The test should not be performed before 2 weeks of age.
- If a GnRH test is planned, this should be carried out before the HCG test (or > 6 weeks after) if as hCG has a long half-life.

Side Effects

- Headaches and/or tiredness are reported side effects.

Preparation

- None required.

Protocol

3 Week Protocol:

Described in table below

1. **Day 1** - Between 8.00a.m and 9.00a.m collect baseline blood samples for testosterone (also androstenedione and dihydrotestosterone if a steroid biosynthetic defect is suspected).

2. Immediately following collection of baseline blood samples, ***give human chorionic gonadotrophin as follows:***

Generic	***Route***	***Dose***	***Frequency***
human chorionic gonadotrophin	*i.m*	*500 units if weight < 5kg* *1000 units if weight 5 - 10kg* *1500 units if weight 10 - 15kg* *3000 units if weight above 15kg*	*Bolus on days 1, 4, 8, 11, 15 & 18.*

3. **Day 4** - Repeat blood sample for testosterone, dihydrotestosterone and androstenedione (72 hours after human chorionic gonadotrophin injection).

4. Administer human chorionic gonadotrophin and continue to administer human chorionic gonadotrophin twice weekly for the next 2 weeks (see table below).

5. Collect the final blood sample for testosterone, DHT and androstenedione 4 days after the last injection of human chorionic gonadotrophin.

Also document the clinical response in terms of testicular descent and change in phallic size.

Time Points:

Week	1		2		3		4
Day	**Mon**	**Thurs**	**Mon**	**Thurs**	**Mon**	**Thurs**	**Mon**
hCG administration	✓	✓	✓	✓	✓	✓	
Blood Sample for testosterone, DHT, A-dione	✓	✓					✓
Urine Steroid profile		✓					✓

Samples

Testosterone, DHT & Androstenedione 1.2 mL clotted blood (white top)

Urinary Steroid Profiling 24hr timed urine in a plain bottle if indicated (collection after injection usually preferred e.g. 24hr prior to day 4 visit, check with consultant). Random/ 4-hour timed sample less reliable but is acceptable. Collection on day 4 usually preferable to day 1.

Interpretation

The normal testosterone response depends on the age of the patient. In infancy, a normal testosterone increment after hCG may vary from 2-fold to 10- or even 20-fold. During childhood, the increment is between 2- and 9-fold. During puberty, as the basal concentration is higher, the increment is less, i.e. 2- to 3-fold.

An absent response with an exaggerated LH/FSH response to LHRH stimulation indicates primary gonadal failure or anorchia. If there is a defect in testosterone biosynthesis, there will be an increase in precursor steroid secretion following HCG stimulation. There are reported errors in the interpretation of the hCG stimulation test in boys ~8yrs of age with increased Testosterone: DHT in the 5α-reductase range. A 5- to 10-fold increment from the basal testosterone constitutes a normal response in the prolonged test.

Samples are sent to Leeds General Infirmary for analysis.
Interpretation (from Leeds):

	Testosterone (nmol/L)	DHT (nmol/L)	Testosterone /DHT ratio post hCG*	Androstenedione (nmol/L)	Androstenedione/ Testosterone ratio post HCG
Normal male adults	8 - 27	0.4-1.9	< 15	1.3 -5.8	<1.0
Normal children (6 months – puberty)	< 0.9	< 0.25	< 15	<1.4	<1.0
5α-reductase deficiency (6 months – puberty)	<0.5	N/A	> 20	N/A	N/A
17-β-hydroxy-steroid dehydrogenase deficiency	N/A	N/A	N/A	N/A	Raised >2.0**

* Testosterone /DHT ratio post hCG 15-20: 5-alpha-reductase deficiency cannot be excluded

** Androstenedione/ Testosterone ratio post HCG: Males, all ages: <1.0 – Likely excludes 17β-hydroxysteroid dehydrogenase deficiency. Adults: >3.0 – Indicative of 17β-hydroxysteroid dehydrogenase deficiency.

References

1. Maimoun L., Philibert P., Cammas B., Audran F., Bouchard P., Fenichel P., Cartigny M., Pienkowski C., Polak M., Skordis N., Mazen I., Ocal G., Berberoglu M., Reynaud R., Baumann C., Cabrol S., Simon D., Kayemba-Kay's K., De Kerdanet M., Kurtz F., Leheup B., Heinrichs C., Tenoutasse S., Van Viet G., Gruters A., Eunice M., Ammini A.C., Hafez M., Hochberg Z., Einaudi S., Mawlawi H.A., del Valle Nunez C.J., Servant N., Lumbroso S., Paris F. & Sultan C. (2011) 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
3. Leeds Children's Hospital Paediatric Endocrinology Dynamic Function Tests: Valid Jan 2022 to Jan 2025. hCG Stimulation Test

Oral Glucose Tolerance Test

Test Name: GLUCOSE TOLERANCE TEST

Principle

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, 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 altered insulin sensitivity in these circumstances.
- This test should also not be performed on patients with hypokalaemic periodic paralysis.
- Do not perform this test at the same time a synacthen test. However, the oral glucose tolerance may be performed after the synacthen test.

Side 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

1. Ensure the patient's fasting blood glucose concentration, checked with a capillary blood sample obtained by finger prick testing with a glucometer, is ≤7.0 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.

2. Prepare the glucose load using **ONE** of the following:
   - ***POLYCAL® (Nutricia Clinical) liquid*** **(contains 0.66g anhydrous glucose per mL; 1.51 mL = 1g anhydrous glucose):** Dose of POLYCAL must be adjusted for the weight at a dose of 2.64 mL POLYCAL/kg body weight (maximum dose 113 mL POLYCAL, equivalent to a 75g glucose load). Add water to make up to a volume of 200 mL.

OR

- ***Glucose tolerance test (Rapilose) Solution:*** Contains 75g anhydrous glucose in 300 mL. For children weighing less than 43kg, the dose is 7 mL (1.75g anhydrous glucose)/kg body weight. The total dose should not exceed 75g anhydrous glucose. If the volume is less than 200 mL, add water to make up to 200 mL.

4. Take a basal sample for glucose (t = 0). Write "t=0" on the tube of blood and time of sampling. Besides sending the sample to lab, use the sample to check bedside point of care blood glucose and record it in the notes.

5. The child should drink the glucose load over a period of no more than 5 min.

6. Take a further blood sample 2hrs (120 min) after finishing the glucose drink. Write "t=120" on the tube of blood and time of sampling. Besides sending the sample to lab, use the sample to check bedside point of care blood glucose and record it in the notes.

Samples

Glucose 1.2 mL fluoride oxalate tube (yellow top).
A drop of blood for bedside blood glucose measurement.

If it is impossible to collect a venous sample, then 0.5 mL (minimum) capillary blood in a fluoride tube may be substituted but the result interpretation is different (see table below). Samples taken at 0 and 120 min must always be the same type.

Interpretation

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

Venous plasma:

- A fasting glucose level of >7.0 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

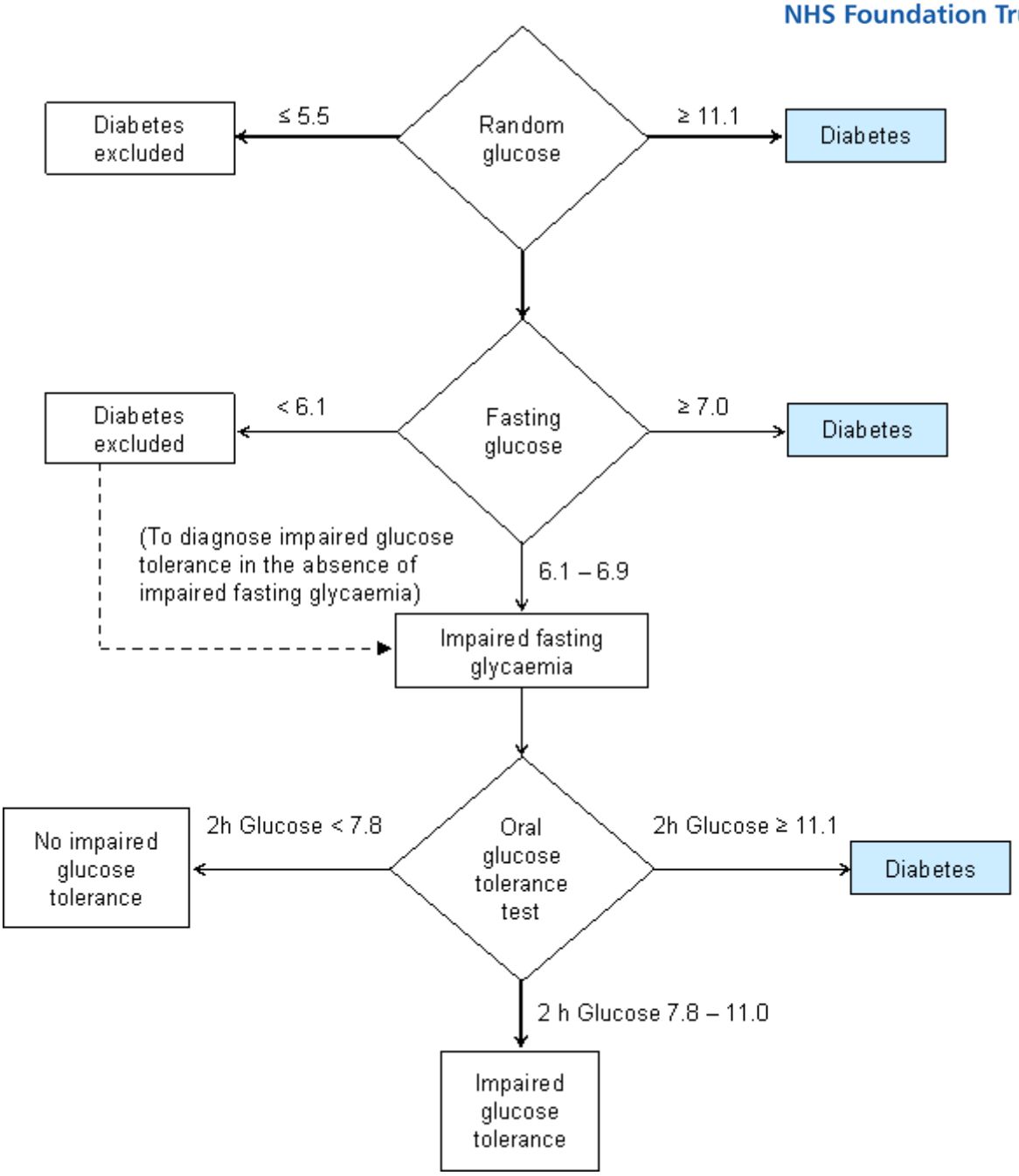

Diagram taken from Brooks *et al* 2005.

References

1. East Kent Hospitals University NHS Foundation Trust Clinical Biochemistry: OGTT – Protocol for paediatrics
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 hyperglycaemia
4. Brooks C., Clayton P. & Brown R. (2005) Brook's clinical paediatric endocrinology, 5th edition. Blackwell publishing, Oxford.

Oral Glucose Tolerance Test with insulin

Test Name: CHILD GLUCOSE TOLERANCE TEST WITH INSULIN DFT

Principle

The increasing prevalence of obese children has also resulted in an increased number of children at risk of developing insulin resistance, which can lead to hyperinsulinaemia and eventually type 2 diabetes mellitus. In contrast to the diagnostic assessment of diabetes, the diagnosis of insulin resistance is less clear, depending on the given situation of an individual. There are no clear criteria to define insulin resistance in children at present.

Indication

- Obese patients with clinical signs of insulin resistance (acanthosis nigricans) and/or a family history of diabetes.

Precautions

- 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
- This test should not be performed on patients who are under physical stress e.g., post surgery, trauma or infection or extreme psychological stress as these may give misleading results.
- This test should not be performed on patients with hypokalaemic periodic paralysis.
- Do not perform this test at the same time a synacthen test. However, the oral glucose tolerance may be performed after the synacthen test.

Side 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).
- OGTT must NOT be performed if the fasting capillary (finger prick) or venous blood glucose concentration is > 7.0 mmol/L or a random glucose > 11.0 mmol/L.
- 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.
- 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

1. Ensure the patient's fasting blood glucose concentration, checked with a capillary blood sample obtained by finger prick testing with a glucometer, is ≤7.0 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.

2. Prepare the glucose load using **ONE** of the following:
   - ***POLYCAL® (Nutricia Clinical) liquid*** **(contains 0.66g anhydrous glucose per mL; 1.51 mL = 1g anhydrous glucose):** Dose of POLYCAL must be adjusted for the weight at a dose of 2.64 mL POLYCAL/kg body weight (maximum dose 113 mL POLYCAL, equivalent to a 75g glucose load). Add water to make up to a volume of 200 mL.

OR

- ***Glucose tolerance test (Rapilose) Solution:*** Contains 75g anhydrous glucose in 300 mL. For children weighing less than 43kg, the dose is 7 mL (1.75g anhydrous glucose)/kg body weight. The total dose should not exceed 75g anhydrous glucose. If the volume is less than 200 mL, add water to make up to 200 mL.

3. Take a basal sample for glucose and insulin (t = 0). Write t = 0 on the tube of blood. Besides sending the sample to lab, use the sample to check bedside point of care blood glucose and record it in the notes.
4. The child should drink the glucose load over a period of about 5 min.
5. Take further blood samples for glucose and insulin at 120 min after finishing the glucose drink and record the sampling time on the tubes. Besides sending the sample to lab, use the sample to check bedside point of care blood glucose and record it in the notes.

Time Points:

Time (min)	Procedure	Blood Samples
0	Take blood then administer glucose load	Glucose, Insulin, POCT glucose
120	-	Glucose, Insulin, POCT glucose

Samples

Glucose 1.2 mL fluoride oxalate tube (yellow top). A drop of blood for bedside blood glucose measurement.

Insulin 1 mL lithium heparin (orange top)

On completion of the test, immediately send all the samples together to the laboratory. The insulin samples must reach the lab within 4 hours of collection.

Interpretation

The 2010 consensus statement recommends there is no clear cut-off to define insulin resistance in children and surrogate measures such as fasting insulin are not ideal.

The following cut-off values taken from SPEG[2] provide useful guidance:

- Fasting insulin is <60 pmol/L in pre-pubertal children or children younger than 10 years or <120 pmol/L in children post pubertal children.
- Peak during the test is normally <600pmol/L.
- Fasting insulin of 120 - 300 pmol/L or peak insulin of 600 - 1800 pmol/L is suggestive of mild to moderate insulin resistance.
- Fasting insulin of >300 pmol/L or peak insulin of >1800 pmol/L is suggestive of severe insulin resistance.

References

1. Levy-Marchal C., Arslanian S., Cutfield W., Sinaiko A., Druet C., Marcovecchio M.L., Chiarelli F. & the Insulin Resistance in Children Consensus Conference Group (2010) Insulin resistance in children: Consensus, perspective and future directions. JCEM 95(12): 5189 – 5198.
2. Managed clinical network of Scottish Paediatric Endocrine Group (SPEG MCN) Dynamic function test handbook for Clinicians January 2012

8 Hour Controlled Fast

Test Name: 8 hour controlled fast DFT

Please discuss with Metabolic/Endocrine Team before carrying out this test. This fast is designed primarily for the use of the Metabolic and Endocrine teams at RMCH. All patients, prior to any fasting studies, should have been assessed and fat oxidation disorders, such as MCAD deficiency, excluded by appropriate investigations.

Management of hypoglycaemia

If at any time during the fast the child becomes hypoglycaemic with bedside finger prick glucometer reading ≤ 2.6 mmol/L (but remember blood glucose [BG] levels are inaccurate at low levels) or if symptomatic, do the following without delay:

- **take blood samples as listed as for the end of the fast (8 hours)** and
- stop the fast immediately after the blood has been taken.
  - If symptomatic, give glucose (3 mL/kg of i.v. 10% glucose) - **INFORM DOCTOR**
  - Give feed if able to tolerate, if not intravenous maintenance fluids, 10% glucose + sodium chloride (e.g. 10% glucose/0.45% sodium chloride)
  - Recheck finger prick BG every 15 min until glucose >4.0mmol/L
  - If BG remains low consider further bolus and increase glucose concentration/ fluid rate (INFORM DOCTOR)
  - CONTACT METABOLIC/ENDOCRINE CONSULTANT ON CALL IF ANY CONCERNS

Protocol

Duration:

AGE	**<6 mo**	6-8 mo	8-12 mo	1-2 yr	2-7 yr	>7 yr
DURATION	**8 hr**	12 hr	16 hr	18 hr	20 hr	24 hr

- Note: These times are for guidance only; hypoglycaemia may develop earlier, particularly if there is an underlying disorder. Please confirm the length of fast with either the metabolic/endocrine team. Careful monitoring of blood glucose is essential throughout the fast. Please record the patient's clinical condition during the fast in his/her notes. This protocol is for an **8 hour fast** (other protocols are available for fasts of different lengths).
- The child should be admitted at around **8 am** on a weekday (not Friday). The child should be clerked in by a member of the endocrine/metabolic team and all blood and urine forms should be printed off at the start of the fast.
- The fast should normally start at **9 am** (after breakfast/snack) and aim to end at **5 pm** on the same day. This allows the collection of the majority of samples when the endocrine/metabolic team is present and when the laboratories are open. Adequate handover should be given to the on call team for the risk of hypoglycaemia if inadvertently the fast ends after 5 pm.
- The patient should stay for 4-6 hours after fast is completed to ensure they remain well and there are no hypoglycaemic episodes. If necessary, an additional overnight stay may be needed to ensure clinical stability.
- Finger prick blood glucose should be monitored by a ward bedside monitor hourly throughout the duration of the fast. If BG < 3.0 mmol/L, check again in 15 minutes. If BG < 2.6mmol/L or if the child is symptomatic of hypoglycaemia (feels hot, sweaty, flushed, tachycardia, decreasing consciousness), a venous sample for glucose and other hypoglycaemic screen bloods should be taken immediately and hypoglycaemia treated as above. At the same time ketones should be measured on the ward blood glucose meter.

Samples

Arrange bottles prior to test. Check the nearest source of ice and ensure availability at the time of hypoglycaemia. Insert the largest possible venous cannula at the start of the fast. Collect blood samples as shown below. All bottles must have the date and time of sample collection recorded.

The samples are listed in order of priority, the most important being Glucose/Lactate, Insulin/C-peptide and FFAs.

Blood Samples		**Time (hr)**			
		0	**4**	**8**	**1 hr post fast**
	Bedside Blood Glucose/ketones	1 hourly monitoring from time of stopping feeds, when/if hypoglycaemia occurs and at end of fast			
1.2 mL fluoride oxalate (yellow)	Glucose/Lactate	✔	✔	✔	✔
1.2 mL heparinised (orange), to biochemistry lab immediately	Insulin/ C-peptide			✔	
1.2 mL heparinised (orange), to biochemistry lab immediately on ice	3 OH butyrate/FFA	✔		✔	
Blood spot cards (or 1.2 mL heparinised sample) to Willink Lab	Acylcarnitines,	✔		✔	
1.2 mL EDTA (pink) (to biochemistry lab immediately, on ice)	Ammonia			✔	✔
Capillary tube	Venous Gas			✔	
1.2 mL clotted (white top) to biochemistry lab	Growth hormone/ Cortisol			✔	

+at end of fast or at time of hypoglycaemia

Urine samples:

5-10 ml in a sterile container	Organic acids & amino acids	Collect aliquots of all urine passed from beginning of fast till 4 hours after end of fast

Glucagon Test

Only To Be Performed If Requested By The Endocrine Team.

- Once fast is completed and there is no evidence of hypoglycaemia: administer 0.03 mg/kg (maximum 1 mg) of Glucagon i.m.
- Blood glucose should be measured at 0, 5, 15, 30,45, 60 and 90 minutes; Blood lactate should be measured at 0, 30, 60 and 90 minutes

Interpretation of glucagon test

Normal children will exhibit a rise in BG of 2 mmol/L. There is an exaggerated response in children with hyperinsulinism.

References

Diagnostics of endocrine function in children and adolescents. Third Edition, Ranke, M.B.; page 30

Controlled fast chart

Name	
DOB	
Hospital no	
Consultant	
Date	

Record details below:

Time of last feed:

Time	BG	Ketones on ward meter	Samples collected	Comments

12 Hour Controlled Fast

Test Name: 12 hour controlled fast DFT

Please discuss with Metabolic/Endocrine Team before carrying out this test. This fast is designed primarily for the use of the Metabolic and Endocrine teams at RMCH. All patients, prior to any fasting studies, should have been assessed and fat oxidation disorders, such as MCAD deficiency, excluded by appropriate investigations.

Management of hypoglycaemia

If at any time during the fast the child becomes hypoglycaemic with bedside finger prick glucometer reading ≤ 2.6 mmol/L (but remember blood glucose [BG] levels are inaccurate at low levels) or if symptomatic, do the following without delay:

- **take blood samples as listed as for the end of the fast (12 hours)** and
- stop the fast immediately after the blood has been taken.
  - If symptomatic, give glucose (3 mL/kg of i.v. 10% glucose) - **INFORM DOCTOR**
  - Give feed if able to tolerate, if not intravenous maintenance fluids, 10% glucose + sodium chloride (e.g. 10% glucose/0.45% sodium chloride)
  - Recheck finger prick BG every 15 min until glucose >4.0mmol/L
  - If BG remains low consider further bolus and increase glucose concentration/ fluid rate (INFORM DOCTOR)
  - CONTACT METABOLIC/ENDOCRINE CONSULTANT ON CALL IF ANY CONCERNS

Protocol

Duration:

AGE	<6 mo	**6-8 mo**	8-12 mo	1-2 yr	2-7 yr	>7 yr
DURATION	8 hr	**12 hr**	16 hr	18 hr	20 hr	24 hr

- Note: These times are for guidance only; hypoglycaemia may develop earlier, particularly if there is an underlying disorder. Please confirm the length of fast with either the metabolic/endocrine team. Careful monitoring 5of blood glucose is essential throughout the fast. Please record the patient's clinical condition during the fast in his/her notes. This protocol is for a **12 hour fast** (other protocols are available for fasts of different lengths).
- The child should be admitted at around **12 noon** on a weekday (not Friday). The child should be clerked in by a member of the endocrine/metabolic team and all blood and urine forms should be printed off at the start of the fast.
- The fast should normally start at **10 pm** (after supper/snack) and aim to end at **10 am** on the following day. This minimises the risk of hypoglycaemia during the night and allows the collection of the majority of samples when the endocrine/metabolic team is present and when the laboratories are open. Adequate handover should be given to the on call team for the risk of hypoglycaemia and if inadvertently the fast ends after 5 pm. The timing of the fast may need to be reviewed if children are usually unable to go through the night without additional food.
- The patient should stay for 4-6 hours after fast is completed to ensure they remain well and there are no hypoglycaemic episodes. If necessary, an additional overnight stay may be needed to ensure clinical stability.
- Finger prick blood glucose should be monitored by a ward bedside monitor hourly throughout the duration of the fast. If BG < 3.0 mmol/L, check again in 15 minutes. If BG < 2.6mmol/L or if the child is symptomatic of hypoglycaemia (feels hot, sweaty, flushed, tachycardia, decreasing consciousness), a venous sample for glucose and other hypoglycaemic screen bloods should be taken immediately and hypoglycaemia treated as above. At the same time ketones should be measured on the ward blood glucose meter.

Samples

Arrange bottles prior to test. Check the nearest source of ice and ensure availability at the time of hypoglycaemia. Insert the largest possible venous cannula at the start of the fast. Collect blood samples as shown below. All bottles must have the date and time of sample collection recorded.

The samples are listed in order of priority, the most important being Glucose/Lactate, Insulin/C-peptide and FFAs.

Blood Samples		Time (hr)					
		0	4	8	10	12+	1 hr post fast
	Bedside Blood Glucose/ketones	1 hourly monitoring from time of stopping feeds, when/if hypoglycaemia occurs and at end of fast					
1.2 mL fluoride oxalate (yellow)	Glucose/Lactate	✔	✔	✔	✔	✔	✔
1.2 mL heparinised (orange), to biochemistry lab immediately	Insulin/ C-peptide					✔	
1.2 mL heparinised (orange), to biochemistry lab immediately on ice	3 OH butyrate/FFA	✔		✔		✔	
Blood spot cards (or 1.2 mL heparinised sample) to Willink Lab	Acylcarnitines,	✔				✔	
1.2 mL EDTA (pink) (to biochemistry lab immediately, on ice)	Ammonia					✔	✔
Capillary tube	Venous Gas					✔	
1.2 mL clotted (white top) to biochemistry lab	Growth hormone/ Cortisol					✔	

+at end of fast or at time of hypoglycaemia

Urine samples:

5-10 ml in a sterile container	Organic acids & amino acids	Collect aliquots of all urine passed from beginning of fast till 4 hours after end of fast

Glucagon Test

***Only* To Be Performed If Requested By The Endocrine Team.**

- Once fast is completed and there is no evidence of hypoglycaemia: administer 0.03 mg/kg (maximum 1 mg) of Glucagon i.m.
- Blood glucose should be measured at 0, 5, 15, 30,45, 60 and 90 minutes; Blood lactate should be measured at 0, 30, 60 and 90 minutes

Interpretation of glucagon test

Normal children will exhibit a rise in BG of 2 mmol/L. There is an exaggerated response in children with hyperinsulinism.

References

Diagnostics of endocrine function in children and adolescents. Third Edition, Ranke, M.B.; page 308

Controlled fast chart

Name	
DOB	
Hospital no	
Consultant	
Date	

Record details below:

Time of last feed:

Time	BG	Ketones on ward meter	Samples collected	Comments

16 Hour Controlled Fast

Test Name: 16 hour controlled fast DFT

Please discuss with Metabolic/Endocrine Team before carrying out this test. This fast is designed primarily for the use of the Metabolic and Endocrine teams at RMCH. All patients, prior to any fasting studies, should have been assessed and fat oxidation disorders, such as MCAD deficiency, excluded by appropriate investigations.

Management of hypoglycaemia

If at any time during the fast the child becomes hypoglycaemic with bedside finger prick glucometer reading ≤ 2.6 mmol/L (but remember blood glucose [BG] levels are inaccurate at low levels) or if symptomatic, do the following without delay:

- **take blood samples as listed as for the end of the fast (16 hours)** and
- stop the fast immediately after the blood has been taken.
  - If symptomatic, give glucose (3 mL/kg of i.v. 10% glucose) - **INFORM DOCTOR**
  - Give feed if able to tolerate, if not intravenous maintenance fluids, 10% glucose + sodium chloride (e.g. 10% glucose/0.45% sodium chloride)
  - Recheck finger prick BG every 15 min until glucose >4.0mmol/L
  - If BG remains low consider further bolus and increase glucose concentration/ fluid rate (INFORM DOCTOR)
  - CONTACT METABOLIC/ENDOCRINE CONSULTANT ON CALL IF ANY CONCERNS

Protocol

Duration:

AGE	<6 mo	6-8 mo	**8-12 mo**	1-2 yr	2-7 yr	>7 yr
DURATION	8 hr	12 hr	**16 hr**	18 hr	20 hr	24 hr

- Note: These times are for guidance only; hypoglycaemia may develop earlier, particularly if there is an underlying disorder. Please confirm the length of fast with either the metabolic/endocrine team. Careful monitoring of blood glucose is essential throughout the fast. Please record the patient's clinical condition during the fast in his/her notes. This protocol is for a **16 hour fast** (other protocols are available for fasts of different lengths).
- The child should be admitted at around **12 noon** on a weekday (not Friday). The child should be clerked in by a member of the endocrine/metabolic team and all blood and urine forms should be printed off at the start of the fast.
- The fast should normally start at **6 pm** (after dinner) and aim to end at **10 am** on the following day. This minimises the risk of hypoglycaemia during the night and allows the collection of the majority of samples when the endocrine/metabolic team is present and when the laboratories are open. Adequate handover should be given to the on call team for the risk of hypoglycaemia and if inadvertently the fast ends after 5 pm. The timing of the fast may need to be reviewed if children are usually unable to go through the night without additional food.
- The patient should stay for 4-6 hours after fast is completed to ensure they remain well and there are no hypoglycaemic episodes. If necessary, an additional overnight stay may be needed to ensure clinical stability.
- Finger prick blood glucose should be monitored by a ward bedside monitor hourly throughout the duration of the fast. If BG < 3.0 mmol/L, check again in 15 minutes. If BG < 2.6mmol/L or if the child is symptomatic of hypoglycaemia (feels hot, sweaty, flushed, tachycardia, decreasing consciousness), a venous sample for glucose and other hypoglycaemic screen bloods should be taken immediately and hypoglycaemia treated as above. At the same time ketones should be measured on the ward blood glucose meter.

Samples

Arrange bottles prior to test. Check the nearest source of ice and ensure availability at the time of hypoglycaemia. Insert the largest possible venous cannula at the start of the fast. Collect blood samples as shown below. All bottles must have the date and time of sample collection recorded.

The samples are listed in order of priority, the most important being Glucose/Lactate, Insulin/C-peptide and FFAs.

Blood Samples		Time (hr)							
		0	4	8	10	12	14	16+	1 hr post fast
	Bedside Blood Glucose/ketones	1 hourly monitoring from time of stopping feeds, when/if hypoglycaemia occurs and at end of fast							
1.2 mL fluoride oxalate (yellow)	Glucose/Lactate	✔	✔	✔	✔	✔	✔	✔	✔
1.2 mL heparinised (orange), to biochemistry lab immediately	Insulin/ C-peptide							✔	
1.2 mL heparinised (orange), to biochemistry lab immediately on ice	3 OH butyrate/FFA	✔				✔		✔	
Blood spot cards (or 1.2 mL heparinised sample) to Willink Lab	Acylcarnitines,	✔						✔	
1.2 mL EDTA (pink) (to biochemistry lab immediately, on ice)	Ammonia							✔	✔
Capillary tube	Venous Gas							✔	
1.2 mL clotted (white top) to biochemistry lab	Growth hormone/ Cortisol							✔	

+at end of fast or at time of hypoglycaemia

Urine samples:

5-10 ml in a sterile container	Organic acids & amino acids	Collect aliquots of all urine passed from beginning of fast till 4 hours after end of fast

Glucagon Test

Only To Be Performed If Requested By The Endocrine Team.

- Once fast is completed and there is no evidence of hypoglycaemia: administer 0.03 mg/kg (maximum 1 mg) of Glucagon i.m.
- Blood glucose should be measured at 0, 5, 15, 30,45, 60 and 90 minutes; Blood lactate should be measured at 0, 30, 60 and 90 minutes

Interpretation of glucagon test

Normal children will exhibit a rise in BG of 2 mmol/L. There is an exaggerated response in children with hyperinsulinism.

References

Diagnostics of endocrine function in children and adolescents. Third Edition, Ranke, M.B.; page 308

Controlled fast chart

Name	
DOB	
Hospital no	
Consultant	
Date	

Record details below:

Time of last feed:

Time	BG	Ketones on ward meter	Samples collected	Comments

18 Hour Controlled Fast

Test Name: 18 hour controlled fast DFT

Please discuss with Metabolic/Endocrine Team before carrying out this test. This fast is designed primarily for the use of the Metabolic and Endocrine teams at RMCH. All patients, prior to any fasting studies, should have been assessed and fat oxidation disorders, such as MCAD deficiency, excluded by appropriate investigations.

Management of hypoglycaemia

If at any time during the fast the child becomes hypoglycaemic with bedside finger prick glucometer reading ≤ 2.6 mmol/L (but remember blood glucose [BG] levels are inaccurate at low levels) or if symptomatic, do the following without delay:

- **take blood samples as listed as for the end of the fast (18 hours)** and
- stop the fast immediately after the blood has been taken.
  - If symptomatic, give glucose (3 mL/kg of i.v. 10% glucose) - **INFORM DOCTOR**
  - Give feed if able to tolerate, if not intravenous maintenance fluids, 10% glucose + sodium chloride (e.g. 10% glucose/0.45% sodium chloride)
  - Recheck finger prick BG every 15 min until glucose >4.0mmol/L
  - If BG remains low consider further bolus and increase glucose concentration/ fluid rate (INFORM DOCTOR)
  - CONTACT METABOLIC/ENDOCRINE CONSULTANT ON CALL IF ANY CONCERNS

Protocol

Duration:

AGE	<6 mo	6-8 mo	8-12 mo	**1-2 yr**	2-7 yr	>7 yr
DURATION	8 hr	12 hr	16 hr	**18 hr**	20 hr	24 hr

- Note: These times are for guidance only; hypoglycaemia may develop earlier, particularly if there is an underlying disorder. Please confirm the length of fast with either the metabolic/endocrine team. Careful monitoring of blood glucose is essential throughout the fast. Please record the patient's clinical condition during the fast in his/her notes. This protocol is for an **18 hour fast** (other protocols are available for fasts of different lengths).

- The child should be admitted at around **12 noon** on a weekday (not Friday). The child should be clerked in by a member of the endocrine/metabolic team and all blood and urine forms should be printed off at the start of the fast.

- The fast should normally start at **6 pm** (after dinner) and aim to end at **12 noon** on the following day. This minimises the risk of hypoglycaemia during the night and allows the collection of the majority of samples when the endocrine/metabolic team is present and when the laboratories are open. Adequate handover should be given to the on call team for the risk of hypoglycaemia and if inadvertently the fast end after 5 pm. The timing of the fast may need to be reviewed if children are usually unable to go through the night without additional food.

- The patient should stay for 4-6 hours after fast is completed to ensure they remain well and there are no hypoglycaemic episodes. If necessary, an additional overnight stay may be needed to ensure clinical stability.

- Finger prick blood glucose should be monitored by a Nova ward bedside monitor hourly throughout the duration of the fast. If BG < 3.0 mmol/L, check again in 15 minutes. If BG <2.6mmol/L or if the child is symptomatic of hypoglycaemia (feels hot, sweaty, flushed, tachycardia, decreasing

consciousness), a venous sample for glucose and other hypoglycaemic screen bloods should be taken immediately and hypoglycaemia treated as above. At the same time ketones should be measured on the ward blood glucose meter.

Samples

Arrange bottles prior to test. Check the nearest source of ice and ensure availability at the time of hypoglycaemia. Insert the largest possible venous cannula at the start of the fast. Collect blood samples as shown below. All bottles must have the date and time of sample collection recorded.

The samples are listed in order of priority, the most important being Glucose/Lactate, Insulin/C peptide and FFAs.

Blood Samples		Time (hr)								
		0	4	8	10	12	14	16	18+	1 hr post fast
	Bedside Blood glucose/ketones	1 hourly monitoring from time of stopping feeds, when/if hypoglycaemia occurs and at end of fast								
1.2 mL fluoride oxalate (yellow)	Glucose/Lactate	✔	✔	✔	✔	✔	✔	✔	✔	✔
1.2 mL heparinised (orange), to biochemistry lab immediately	Insulin/ C-peptide								✔	
1.2 mL heparinised (orange), to biochemistry lab immediately on ice	3 OH butyrate/FFA	✔					✔		✔	
Blood spot cards (or 1.2 mL heparinised sample) to Willink Lab	Acylcarnitines,	✔							✔	
1.2 mL EDTA (pink) (to biochemistry lab immediately, on ice)	Ammonia								✔	✔
Capillary tube	Venous Gas								✔	
1.2 mL clotted (white top) to biochemistry lab	Growth hormone/ Cortisol								✔	

+at end of fast or at time of hypoglycaemia

Urine samples:

5-10 mL in a sterile container	Organic acids & amino acids	Collect aliquots of all urine passed from beginning of fast till 4 hours after end of fast

Glucagon Test

Only To Be Performed If Requested By The Endocrine Team.

- Once fast is completed and there is no evidence of hypoglycaemia: administer 0.03 mg/kg (maximum 1 mg) of Glucagon i.m..
- Blood glucose should be measured at 0, 5, 15, 30,45, 60 and 90 minutes; Blood lactate should be measured at 0, 30, 60 and 90 minutes

Interpretation of glucagon test

Normal children will exhibit a rise in BG of 2 mmol/L. There is an exaggerated response in children with hyperinsulinism.

References

Diagnostics of endocrine function in children and adolescents. Third Edition, Ranke, M.B.; page 308

Controlled fast chart

Name	
DOB	
Hospital no	
Consultant	
Date	

Record details below:

Time of last feed:

Time	BG	Ketones on ward meter	Samples collected	Comments

20 Hour Controlled Fast

Test Name: 20 hour controlled fast DFT

Please discuss with Metabolic/Endocrine Team before carrying out this test. This fast is designed primarily for the use of the Metabolic and Endocrine teams at RMCH. All patients, prior to any fasting studies, should have been assessed and fat oxidation disorders, such as MCAD deficiency, excluded by appropriate investigations.

Management of hypoglycaemia

If at any time during the fast the child becomes hypoglycaemic with bedside finger prick glucometer reading ≤ 2.6 mmol/L (but remember blood glucose [BG] levels are inaccurate at low levels) or if symptomatic, do the following without delay:

- **take blood samples as listed as for the end of the fast (20 hours)** and
- stop the fast immediately after the blood has been taken.
  - If symptomatic, give glucose (3 mL/kg of i.v. 10% glucose) - **INFORM DOCTOR**
  - Give feed if able to tolerate, if not intravenous maintenance fluids, 10% glucose + sodium chloride (e.g. 10% glucose/0.45% sodium chloride)
  - Recheck finger prick BG every 15 min until glucose >4.0 mmol/L
  - If BG remains low consider further bolus and increase glucose concentration/ fluid rate (INFORM DOCTOR)
  - CONTACT METABOLIC/ENDOCRINE CONSULTANT ON CALL IF ANY CONCERNS

Protocol

Duration:

AGE	<6 mo	6-8 mo	8-12 mo	1-2 yr	**2-7 yr**	>7 yr
DURATION	8 hr	12 hr	16 hr	18 hr	**20 hr**	24 hr

- Note: These times are for guidance only; hypoglycaemia may develop earlier, particularly if there is an underlying disorder. Please confirm the length of fast with either the metabolic/endocrine team. Careful monitoring of blood glucose is essential throughout the fast. Please record the patient's clinical condition during the fast in his/her notes. This protocol is for a **20 hour fast** (other protocols are available for fasts of different lengths).

- The child should be admitted at around **12 noon** on a weekday (not Friday). The child should be clerked in by a member of the endocrine/metabolic team and all blood and urine forms should be printed off at the start of the fast.

- The fast should normally start at **4 pm** (after lunch/snack) and aim to end at **12 noon** on the following day. This minimises the risk of hypoglycaemia during the night and allows the collection of the majority of samples when the endocrine/metabolic team is present and when the laboratories are open. Adequate handover should be given to the on call team, for the risk of hypoglycaemia and if inadvertently the fast ends after 5 pm. The timing of the fast may need to be reviewed if children are usually unable to go through the night without additional food.

- The patient should stay for 4-6 hours after fast is completed to ensure they remain well and there are no hypoglycaemic episodes. If necessary, an additional overnight stay may be needed to ensure clinical stability.

- Finger prick blood glucose should be monitored by Nova ward bedside monitor hourly throughout the duration of the fast. If BG < 3.0 mmol/L, check again in 15 minutes. If BG <2.6mmol/L or if the child is symptomatic of hypoglycaemia (feels hot, sweaty, flushed, tachycardia, decreasing consciousness), a venous sample for glucose and other hypoglycaemic screen bloods should be

taken immediately and hypoglycaemia treated as above. At the same time ketones should be measured on the ward blood glucose meter.

Samples

Arrange bottles prior to test. Check the nearest source of ice and ensure availability at the time of hypoglycaemia. Insert the largest possible venous cannula at the start of the fast. Collect blood samples as shown below. All bottles must have the date and time of sample collection recorded.

The samples are listed in order of priority, the most important being Glucose/Lactate, Insulin/C peptide and FFAs.

		Time (hr)									
Blood Samples		**0**	**4**	**8**	**10**	**12**	**14**	**16**	**18**	**20+**	**1 hr post fast**
	Bedside Blood glucose/ketones	1 hourly monitoring from time of stopping feeds, when/if hypoglycaemia occurs and at end of fast									
1.2 mL fluoride oxalate (yellow)(discuss with biochemistry lab before sending)	Glucose/lactate	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔
1.2 mL heparinised (orange), to biochemistry lab immediately on ice	Insulin/ C-peptide									✔	
1.2 mL heparinised (orange), to biochemistry lab immediately	3 OH butyrate/FFA	✔						✔		✔	
Blood spot cards (or 1.2 mL heparinised sample) to Willink Lab	Acylcarnitines	✔								✔	
1.2 mL EDTA (pink) (to biochemistry lab immediately, on ice)	Ammonia									✔	✔
Capillary tube	Venous Gas									✔	
1.2 mL clotted (white top) to biochemistry lab	Growth hormone/ Cortisol									✔	

+at end of fast or at time of hypoglycaemia

Urine samples:

5-10 mL in a sterile container	Organic acids & amino acids	Collect aliquots of all urine passed from beginning of fast till 4 hours after end of fast

Glucagon Test

Only To Be Performed If Requested By The Endocrine Team.

- Once fast is completed and there is no evidence of hypoglycaemia: administer 0.03 mg/kg (maximum 1 mg) of Glucagon i.m.
- Blood glucose should be measured at 0, 5, 15, 30,45, 60 and 90 minutes; Blood lactate should be measured at 0, 30, 60 and 90 minutes

Interpretation of glucagon test

Normal children will exhibit a rise in BG of 2 mmol/L. There is an exaggerated response in children with hyperinsulinism.

References

Diagnostics of endocrine function in children and adolescents. Third Edition, Ranke, M.B.; page 308

Controlled fast chart

Name	
DOB	
Hospital no	
Consultant	
Date	

Record details below:

Time of last feed:

Time	BG	Ketones on ward meter	Samples collected	Comments

24 Hour Controlled Fast

Test Name: 24 hour controlled fast DFT

Please discuss with Metabolic/Endocrine Team before carrying out this test. This fast is designed primarily for the use of the Metabolic and Endocrine teams at RMCH. All patients, prior to any fasting studies, should have been assessed and fat oxidation disorders, such as MCAD deficiency, excluded by appropriate investigations.

Management of hypoglycaemia

If at any time during the fast the child becomes hypoglycaemic with bedside finger prick glucometer reading ≤ 2.6 mmol/L (but remember blood glucose [BG] levels are inaccurate at low levels) or if symptomatic, do the following without delay:

- **take blood samples as listed as for the end of the fast (24 hours)** and
- stop the fast immediately after the blood has been taken.
  - If symptomatic, give glucose (3 mL/kg of i.v. 10% glucose) - **INFORM DOCTOR**
  - Give feed if able to tolerate, if not intravenous maintenance fluids, 10% glucose + sodium chloride (e.g. 10% glucose/0.45% sodium chloride)
  - Recheck finger prick BG every 15 min until glucose >4.0mmol/L
  - If BG remains low consider further bolus and increase glucose concentration/ fluid rate (INFORM DOCTOR)
  - CONTACT METABOLIC/ENDOCRINE CONSULTANT ON CALL IF ANY CONCERNS

Protocol

Duration:

AGE	<6 mo	6-8 mo	8-12 mo	1-2 yr	2-7 yr	**>7 yr**
DURATION	8 hr	12 hr	16 hr	18 hr	20 hr	**24 hr**

- Note: These times are for guidance only; hypoglycaemia may develop earlier, particularly if there is an underlying disorder. Please confirm the length of fast with either the metabolic/endocrine team. Careful monitoring of blood glucose is essential throughout the fast. Please record the patient's clinical condition during the fast in his/her notes. This protocol is for a **24 hour fast** (other protocols are available for fasts of different lengths).
- The child should be admitted at around **8 am** on a weekday (not Friday). The child should be clerked in by a member of the endocrine/metabolic team and all blood and urine forms should be printed off at the start of the fast.
- The fast should normally start at **10 am** (after breakfast/snack) and aim to end at **10 am** on the following day. This allows the collection of the majority of samples when the endocrine/metabolic team is present and when the laboratories are open. Adequate handover should be given to the on call team for the risk of hypoglycaemia and if inadvertently the fast ends after 5 pm. The timing of the fast may need to be reviewed if children are usually unable to go through the night without additional food.
- The patient should stay for 4-6 hours after fast is completed to ensure they remain well and there are no hypoglycaemic episodes. If necessary, an additional overnight stay may be needed to ensure clinical stability.
- Finger prick blood glucose should be monitored by a ward bedside monitor hourly throughout the duration of the fast. If BG < 3.0 mmol/L, check again in 15 minutes. If BG < 2.6mmol/L or if the child is symptomatic of hypoglycaemia (feels hot, sweaty, flushed, tachycardia, decreasing consciousness), a venous sample for glucose and other hypoglycaemic screen bloods should be taken immediately and hypoglycaemia treated as above. At the same time ketones should be measured on the ward blood glucose meter.

Samples

Arrange bottles prior to test. Check the nearest source of ice and ensure availability at the time of hypoglycaemia. Insert the largest possible venous cannula at the start of the fast. Collect blood samples as shown below. All bottles must have the date and time of sample collection recorded.

The samples are listed in order of priority, the most important being Glucose/Lactate, Insulin/C-peptide and FFAs.

		Time (hr)											
Blood Samples		**0**	**4**	**8**	**10**	**12**	**14**	**16**	**18**	**20**	**22**	**24+**	**1 hr post fast**
	Bedside Blood glucose/ketones	1 hourly monitoring from time of stopping feeds, when/if hypoglycaemia occurs and at end of fast											
1.2 mL fluoride oxalate (yellow)	Glucose/lactate	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔
1.2 mL heparinised (orange), to biochemistry lab immediately on ice	Insulin/ C-peptide											✔	
1.2 mL heparinised (orange), to biochemistry lab immediately	3 OH butyrate/FFA	✔								✔		✔	
Blood spot cards (or 1.2 mL heparinised sample) to Willink Lab	Acylcarnitines	✔										✔	
1.2 mL EDTA (pink) (to biochemistry lab immediately, on ice)	Ammonia											✔	✔
Capillary tube	Venous Gas											✔	
1.2 mL clotted (white top) to biochemistry lab	Growth hormone/ Cortisol											✔	

+at end of fast or at time of hypoglycaemia

Urine samples:

5-10 ml in a sterile container	Organic acids & amino acids	Collect aliquots of all urine passed from beginning of fast till 4 hours after end of fast

Glucagon Test

Only To Be Performed If Requested By The Endocrine Team.

- Once fast is completed and there is no evidence of hypoglycaemia: administer 0.03 mg/kg (maximum 1 mg) of Glucagon i.m.
- Blood glucose should be measured at 0, 5, 15, 30,45, 60 and 90 minutes; Blood lactate should be measured at 0, 30, 60 and 90 minutes

Interpretation of glucagon test

Normal children will exhibit a rise in BG of 2 mmol/L. There is an exaggerated response in children with hyperinsulinism.

References

Diagnostics of endocrine function in children and adolescents. Third Edition, Ranke, M.B.; page 308

Controlled fast chart

Name	
DOB	
Hospital no	
Consultant	
Date	

Record details below:

Time of last feed:

Time	BG	Ketones on ward meter	Samples collected	Comments

Oral Glucose Loading Test for hypoglycaemia

Test Name: CHILD ORAL GLUCOSE LOADING TEST FOR HYPOGLYCAEMIA DFT

Principle

In contrast to fasting hypoglycaemia, postprandial hypoglycaemia occurs several hours after eating. Some disorders that cause hypoglycaemia predominantly in the postprandial state are the non-insulinoma pancreatogenous hypoglycaemia syndrome (NIPHS), post gastric bypass hypoglycaemia, and insulin autoimmune hypoglycaemia.

Please refer to the metabolic team if glycogen storage disease 0 (GSD0) is suspected.

It is important to appreciate that sometimes patients are unable to differentiate fasting from postprandial occurrences of hypoglycaemia, and some disorders can produce both fasting and postprandial hypoglycaemia.

Indication

Patients who demonstrate unexplained post prandial hypoglycaemia.

Precautions

- 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.
- This test should not be performed on patients who are under physical stress e.g., post-surgery, trauma or infection or extreme psychological stress as these may give misleading results.
- This test should not be performed on patients with hypokalaemic periodic paralysis.

Side Effects

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

Preparation

The patient should be fasted for a period of 3 to 8 h, the time period chosen should be dependent on the patient's age and the patient's usual interval between meals.

Protocol

1. Collect a urine sample prior to starting the test and send for organic acid analysis.
2. Insert an indwelling 22-gauge blue cannula 30 min before the expected start of the test and start the patient on a saline drip.
3. Prepare the glucose load (2 g/kg with a maximum of 50 g), as a 10% solution in water.
4. Take a basal sample for glucose, lactate, beta-hydroxybutyrate and bedside ketones. Write t = 0 on the tube of blood.
5. Administer the glucose load orally or through a nasogastric tube over 5–10 min.
6. Take blood samples at 30, 60, 90, 120, 150, 180 mins.
7. Write the times on the samples.
8. If the bedside glucose drops <2.6 mmol/L at any point – collect the plasma glucose, insulin and bedside ketones samples. Then **stop the test** and treat the hypoglycaemia.

Time Points:

Time (min)	Procedure	Blood Samples
0	Check blood glucose using meter. Take blood samples then administer glucose load.	Plasma glucose, lactate, beta-hydroxybutyrate and insulin
30	Check blood glucose using meter	If bedside glucose is <2.6 mmol/L take the following blood samples: Glucose, insulin, bedside ketones
60	Check blood glucose using meter	If bedside glucose is <2.6 mmol/L take the following blood samples: Glucose, insulin, bedside ketones
90	Check blood glucose using meter	If bedside glucose is <2.6 mmol/L take the following blood samples: Glucose, insulin, bedside ketones
120	Check blood glucose using meter	Plasma glucose and insulin (as normal OGTT protocol). If POCT <2.6 mmol/L take bedside ketones.
150	Check blood glucose using meter	If bedside glucose is <2.6 mmol/L take the following blood samples: Glucose, insulin, bedside ketones
180	Check blood glucose using meter	If bedside glucose is <2.6 mmol/L take the following blood samples: Glucose, lactate, beta-hydroxybutyrate Insulin

Samples

Glucose and lactate 1.2 mL fluoride oxalate (yellow top)

Insulin 1.2 mL lithium heparin (orange top)

The insulin samples must reach the lab within 4 hours of collection

Beta-hydroxybutyrate 1.2 mL lithium heparin (orange top)

The insulin samples will be vetted by the laboratory – only insulin samples that have concurrent laboratory glucose of <3.0 mmol/L will be analysed (except for the baseline and 120 min insulin samples – which will be analysed, as routine OGTT protocol.)

Interpretation

Detectable insulin when blood glucose is <2.5 mmol/L is inappropriate.

References

1. Permutt MA. Postprandiol hypoglycemia. Diabetes. 1976 Aug;25(8):719-33.
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3. Brun JF, Fedou C, Mercier J. Postprandial reactive hypoglycemia. Diabetes Metab 2000; 26:337.
4. Bhattacharya K. Investigation and management of the hepatic glycogen storage diseases. 2015. Transl Pediatr. 2015 Jul;4(3):240-8.

Protein loading test for hypoglycaemia

Test Name: CHILD PROTEIN LOADING TEST FOR HYPOGLYCAEMIA DFT

Please discuss with Endocrine Team before carrying out this test. This fast is designed primarily for the use of the Endocrine teams at RMCH.

Management of hypoglycaemia

If at any time during the fast the child becomes hypoglycaemic with bedside finger prick glucometer reading ≤ 2.6 mmol/L (but remember blood glucose [BG] levels are inaccurate at low levels) or if symptomatic, do the following without delay:

- **take blood samples as listed as for the next time point** and
- stop the test immediately after the blood has been taken.
  - If symptomatic, give glucose (3 mL/kg of i.v. 10% glucose) - **INFORM DOCTOR**
  - Give feed if able to tolerate, if not intravenous maintenance fluids, 10% glucose + sodium chloride (e.g., 10% glucose/0.45% sodium chloride)
  - Recheck finger prick BG every 15 min until glucose >4.0mmol/L
  - If BG remains low, consider further bolus and increase glucose concentration/ fluid rate (INFORM DOCTOR)
  - CONTACT ENDOCRINE CONSULTANT ON CALL IF ANY CONCERNS

Principle

Protein sensitivity is observed in some patients with CHI and is often seen in patients with GLUD1 gain of function mutations (hyperinsulinism/hyperammonaemia HI/HA syndrome). Protein sensitivity is also observed in patients with mutations in *ABCC8* gene encoding the SUR1 protein. **Caution - these patients can become severely hypoglycaemic in response to a protein/leucine load.** This test should only be done after consultation with the consultant looking after the patient. For safety reasons protein is used in the test, not pure leucine. The protein can be administe in the form of a drink (Maxipro/Vitapro) or as steamed fat-free chicken breast.

Indication

Patients suspected of having congenital hyperinsulinism with hyperammonaemia syndrome.

Precautions

- This test should not be performed on patients who are under physical stress e.g., post-surgery, trauma or infection or extreme psychological stress as these may give misleading results.
- This test should not be performed on patients with hypokalaemic periodic paralysis.

Side Effects

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

Preparation

The patient should be fasted for a period of 3 to 8 h, the time period chosen should be dependent on the patient's age and the patient's usual interval between meals. However, this may not be possible with some patients.

Finger prick blood glucose should be monitored by a ward bedside monitor throughout the duration of the test. If BG < 3.0 mmol/L, check again in 15 minutes. If BG < 2.6 mmol/L or if the child is symptomatic of hypoglycaemia (feels hot, sweaty, flushed, tachycardia, decreasing consciousness), a venous sample for glucose, insulin and ammonia should be taken immediately and hypoglycaemia treated as above.

Options for protein loading

For a protein drink - Order Maxipro (1.5g/kg)/ Vitapro from the diet kitchen the day before the test.
For a protein meal - 35 g protein per m^2 surface area as steamed fat-free chicken breast to be eaten within 30 min.

Procedure

Remember to take all necessary precautions when carrying out this test. Treat in a similar manner to a diagnostic fast.

1. At t = 0 measure blood glucose using meter and take blood samples for glucose, insulin and ammonia
2. Administer the protein drink/meal. The drink/meal should be consumed within 30 min.
3. At 30 mins measure the bedside blood glucose. If the bedside glucose is <3.0 mmol/L take blood samples for glucose, insulin and ammonia. Record this information in the chart on the next page and write on t = 30 min on all samples.
4. Repeat this at 60 min, 90 min, 120 min, 150 min and 180 min. At 120 min take blood samples for plasma glucose, insulin and ammonia (as normal OGTT protocol). Even if bedside glucose is not <2.6 mmol/L.
5. If the child becomes hypoglycaemic at any stage of the test, stop and treat the hypoglycaemia.

Time Points:

Time (min)	Procedure	Blood Samples
0	Check blood glucose using meter. Take blood samples then administer protein load (Maxipro/Vitapro or steamed fat-free chicken breast).	Plasma glucose, insulin and ammonia
30	Check blood glucose using meter	If bedside glucose is <2.6 mmol/L take the following blood samples: Plasma glucose, insulin and ammonia
60	Check blood glucose using meter	If bedside glucose is <2.6 mmol/L take the following blood samples: Plasma glucose, insulin and ammonia
90	Check blood glucose using meter	If bedside glucose is <2.6 mmol/L take the following blood samples: Plasma glucose, insulin and ammonia
120	Check blood glucose using meter	Plasma glucose, insulin and ammonia (as normal OGTT protocol).
150	Check blood glucose using meter	If bedside glucose is <2.6 mmol/L take the following blood samples: Plasma glucose, insulin and ammonia
180	Check blood glucose using meter	If bedside glucose is <2.6 mmol/L take the following blood samples: Plasma glucose, insulin and ammonia

Samples

Glucose 1.2 mL fluoride oxalate (yellow top)

Insulin 1.2 mL lithium heparin (orange top)

The insulin samples must reach the lab within 4 hours of collection

Ammonia 1.2 mL EDTA (pink top)

Ammonia is unstable – send sample IMMEDIATELY to laboratory on ice for analysis

Interpretation

Detectable insulin and elevated ammonia when blood glucose is <2.5 mmol/L is inappropriate.

The insulin samples will be vetted by the laboratory – only insulin samples that have concurrent laboratory glucose of <3.0 mmol/L will be analysed – except for the baseline and 120 min samples.

References

1. Fourtner C, Stanley A, Kelly Protein-sensitive hypoglycemia without leucine sensitivity in hyperinsulinism caused by KATP channel mutations. The Journal of Pediatrics, 2006, Volume 149, Issue 1 , Pages 47 - 52.
2. Kelly A, Ng D, Ferry RJ Jr, Grimberg A, Koo-McCoy S, Thornton PS, Stanley CA. Acute insulin responses to leucine in children with the hyperinsulinism/ hyperammonemia syndrome. J Clin Endocrinol Metab. 2001;86(8):3724–3728.
3. Heslegrave A, Kapoor RR, Eaton S, Chadefaux B, Akcay T, Simsek E, Flanagan SE, Ellard S, Hussain K. Leucine-sensitive hyperinsulinaemic hypoglycaemia in patients with loss of function mutations in 3-Hydroxyacyl-CoA Dehydrogenase. Orphanet J Rare Dis. 2012 May 14;7:25

Protein loading test for hypoglycaemia chart

Name	
DOB	
Hospital no	
Consultant	
Date	

Interval (mins)	Time collected	BM (mmol/L)	Glucose (mmol/L)	Insulin (pmol/L)	Ammonia (µmol/L)

]

Tubular Reabsorption of Phosphate

Test Name: TUBULAR REABSORPTION PHOSPHATE (PANEL - URINE AND BLOOD)

Principle

Many factors affect the renal tubular reabsorption of phosphate including PTH, diet, ECF volume, acid-base status. Calculation of the ratio of the renal tubular maximum reabsorption rate of phosphate to glomerular filtration rate (TmP/GFR) is much more useful than measurement of urine phosphate alone. It is independent of GFR and has replaced earlier indices of renal phosphate handling (phosphate excretion index).

Indication

- Investigation of persistent unexplained hypophosphataemia or assessment of renal tubular function.

Precautions

- None

Side Effects

- N/A

Preparation

- The test should ideally be carried out in the morning, after an overnight fast, as there is a significant diurnal variation.
- Stop phosphate supplements prior to the test.

Protocol

1. After waking the patient should empty the bladder – discard urine. The patient may drink water during the test.
2. Collect the next urine passed and send to the lab straight away for acidification. Record the time of collection.
3. Collect a blood sample for U&E, phosphate and creatinine (1.2 mL Lithium Heparin – orange top) and record the time of collection. The ***blood must be collected within 2 hours of the urine sample*****.** Capillary samples are unsuitable due to the effect on phosphate concentration.

Samples

U&E, phosphate and creatinine 1.2 mL lithium heparin (orange top)

Urine phosphate and creatinine 10 mL urine Sarstedt tube

Interpretation

Calculation of TmP/GFR in children

TmP/GFR = Pphosphate - (Uphosphate x Pcreatinine/ Ucreatinine)

P= plasma concentration U= urine concentration
All concentrations in mmol/L

Age	TmP/GFR in mmol/L
Newborns	1.55 – 2.97
1 month – 2 yrs	1.07 – 2.23
2 - 12 yrs	1.10 – 1.88
12 - 16 yrs	0.93 – 1.71
>16 yrs	0.88 – 1.26

In general, when hyperphosphataemia is due to increased phosphate flow from gut, cells or bone there is a decrease in TmP/GFR i.e. a decrease in phosphate reabsorption. Hypophosphataemia due to decreased phosphate flow results in an increased TmP/GFR.

A decreased TmP/GFR is found in hypophosphataemia due to renal tubular dysfunction e.g. Fanconi syndrome, X-linked hypophosphataemic rickets. There is also a renal component to the hypophosphataemia which can follow glucose infusion/refeeding after starvation and respiratory alkalosis, leading to a decreased TmP/GFR, despite a decrease in phosphate flow. Hyperparathyroidism (primary and secondary) can also cause a decrease in TmP/GFR.

Note on use in adults

N.B. Use of this formula in adults may require a correction factor, α, when the TRP is ≥0.86.

TRP = 1- (Uphosphate x Pcreatinine/ Pphosphate x Ucreatinine)

If TRP ≥0.86 then:
α = 0.3 x TRP/ {1-(0.8 x TRP)}
TmP/GFR = α x Pphosphate

If TRP <0.86 then:
TmP/GFR = TRP x Pphosphate (mathematically the same as the equation on the previous page).

References

1. Payne RB. Renal tubular reabsorption of phosphate (TmP/GFR): indications and interpretation. *Ann Clin Biochem* 1998; **35**: 201-206
2. Stark H, Eisenstein B, Tieder M, Rachmel A, Alpert G. Direct measurement of TP/GFR: A simple and reliable parameter of renal phosphate handling. *Nephron* 1986; **22**: 125-128.
3. Brodehl J, Krause A, Hoyer PF. Assessment of maximal tubular phosphate reabsorption: comparison of direct measurement with the nomogram of Bijvoet. *Pediar Nephrol* 1988; **2**: 183-189.
4. Alon U, Hellerstein S. Assessment and interpretation of tubular threshold for phosphate in infants and children. *Pediar Nephrol* 1994; **8**: 250-251