Clinical and laboratory investigation of adult spontaneous hypoglycaemia

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Adult spontaneous hypoglycaemia is not a diagnosis per se but a manifestation of a disease. Although rare, it is important to identify spontaneous hypoglycaemia and its causes because treatment may be preventative or curative. Hypoglycaemia can occur as an epiphenomenon in many serious diseases. It is sufficient to recognise the disease’s association with hypoglycaemia and then take appropriate action to prevent the recurrence of hypoglycaemia. In investigating apparently healthy individuals, common pitfalls to avoid are: failure to recognise subacute neuroglycopenia clinically; failure to document hypoglycaemia adequately during symptoms; failure to measure pancreatic hormones, counter-regulatory hormones, and ketones in hypoglycaemic samples; failure to recognise pre-analytical and analytical limitations of laboratory assays; and failure to abandon obsolete and inappropriate investigations. Providing these caveats are met, appropriate laboratory and radiological investigations will almost always uncover the cause of spontaneous hypoglycaemia.

Hypoglycaemia is not a diagnosis itself, but a manifestation of a disease process. Hypoglycaemia has many causes (table 1), but in practice it is most commonly iatrogenic, and the result of overtreatment of patients with diabetes with insulin or sulphonylureas. This article discusses the clinical and laboratory investigation of spontaneous (non-diabetic) hypoglycaemia in adults, which may be uncommon but important nevertheless, because often preventative or curative treatment is available. The investigation and treatment of neonatal and childhood hypoglycaemia is covered elsewhere.1 2

PATHOPHYSIOLOGY OF HYPOGLYCAEMIA

The autoregulation of pancreatic insulin and glucagon secretion normally maintains circulating glucose between 3.5 mmol/litre and 10 mmol/litre. Insulin secretion, which is stimulated by glucose absorption, returns to basal values within two to four hours as glucose concentrations fall and glucagon secretion rises on completion of glucose absorption.

Homeostatic mechanisms to reverse hypoglycaemia include stimulation of the sympathetic nervous system, and counter-regulatory hormonal responses. The net effect of these is to suppress insulin secretion, promote hunger, increase glucose output by stimulating glycogenolysis and gluconeogenesis, reduce peripheral tissue glucose uptake, and provide alternative fuel sources by promoting lipolysis and ketogenesis.

CLINICAL MANIFESTATIONS OF HYPOGLYCAEMIA

The symptoms of hypoglycaemia are stereotypical and manifested through alteration in cerebral metabolism, and are hence termed neuroglycopenia. There are three distinct neuroglycopenic syndromes; acute, subacute, and chronic neuroglycopenia. Acute neuroglycopenia, most commonly associated with iatrogenic hypoglycaemia, is characterised by sweating, anxiety, tremor, palpitations, tachycardia, pallor, diaphoresis, hunger, and paraesthesias. Subacute neuroglycopenia is most commonly associated with spontaneous hypoglycaemia and is also referred to as hypoglycaemic unawareness in patients with type 1 diabetes mellitus. It presents with episodic disorientation, somnolence, personality changes, amnesia, and loss of consciousness. Clinical features common to both acute and subacute neuroglycopenia include transient hemiplegia, strabismus, hypothermia, hyperthermia, convulsions, and automatism. If untreated, these syndromes may progress to stupor, coma, and even death as a result of cerebral oedema, but fortunately this is rare because of the effectiveness of counter-regulatory hyperglycaemic homeostatic mechanisms. Chronic neuroglycopenia, virtually confined to patients with insulinoma or patients with diabetes who are overtreated with insulin, is rare and presents with insidious progressive mental illness resembling personality disorders, schizophrenia, paranoid

Abbreviations: AIS, autoimmune insulin syndrome; β-OHB, β hydroxybutyrate; CSF, cerebrospinal fluid; GH, growth hormone; IGF, insulin-like growth factor; IRA, antinsulin receptor antibodies; IR, immunoreactive insulin; NICTH, non-islet cell tumour hypoglycaemia

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Accepted for publication 14 February 2003

J Clin Pathol 2003;56:641–646

www.jclinpath.com
Although typical, symptoms of hypoglycaemia are non-specific. Acute and subacute neuroglycopenia can only be confidently confirmed when Whipple’s triad is fulfilled; namely, neuroglycopenic symptoms, a low blood glucose, and symptoms relieved by raising blood glucose to or above normal.

Brain glucose transporter activity adapts to circulating glucose; it is upregulated and downregulated by hypoglycaemia and hyperglycaemia, respectively. This could, in part, explain why chronically hyperglycaemic patients may experience neuroglycopenia at higher glucose concentrations and chronically hypoglycaemic patients may experience it at lower glucose concentrations when compared with normal healthy subjects.

### INVESTIGATION OF HYPOGLYCAEMIA

The investigation of hypoglycaemia involves an index of suspicion, confirmation, or exclusion of hypoglycaemia and its aetiology if it is confirmed. Spontaneous hypoglycaemia should be considered in anyone who presents with an episode or episodic subacute neuroglycopenia, even if there may be an alternative explanation for his or her symptoms. It is desirable that a blood sample should be collected when the patient is symptomatic—first, to confirm or refute hypoglycaemia and second, if confirmed, it offers the ideal and sometimes only opportunity to uncover its underlying aetiology.

Often, however, patients referred for a medical opinion are asymptomatic when seen in the outpatient clinic, at which time their blood glucose concentration is usually unhelpful. In this situation, the options are to attempt to provoke a hypoglycaemic attack, or to obtain a blood sample during symptoms for laboratory measurement of glucose concentrations. Provocation of a hypoglycaemic attack involves fasting, with or without exercise, when fasting hypoglycaemia is suspected, or giving a carbohydrate rich mixed meal when reactive hypoglycaemia is suspected. Other provocative tests are of limited value in the initial investigation of hypoglycaemia because of poor diagnostic specificity and sensitivity. The intravenous tolbutamide test has been used to provoke hypoglycaemia, but is no longer available in the UK. The L-leucine test, intravenous glucagon test, and selective arterial pancreatic calcium stimulation test may each have a limited role in the differential diagnosis of hypoglycaemia, but not in its initial investigation.\(^7\)\(^-\)\(^11\)

Obtaining a blood sample during symptoms entails training the patient, relative, or friend to collect a capillary blood sample into a suitable capillary tube or on to specially prepared filter paper\(^7\) for later laboratory blood glucose measurement, and if hypoglycaemia is confirmed further investigation is obligatory.

#### Provocation tests

##### Overnight fast

Most patients with episodic spontaneous hypoglycaemia will have at least one overnight fasting (18 hours) plasma glucose concentration of < 2.5 mmol/litre, when measured on three separate occasions.\(^5\) The hypoglycaemic episodes may appear asymptomatic, but often they can be shown to be associated with mild impairment of cognitive function if this is specifically sought.

##### Exercise test

Exercise is an important factor in the pathogenesis of insulin induced hypoglycaemia. This is the basis of the exercise test, which is used to precipitate hypoglycaemia in patients with endogenous hyperinsulinism, who might otherwise be able to tolerate prolonged periods of fasting.\(^5\) Blood is collected before and at 10 minute intervals during 30 minutes of intense exercise, and then for 30 minutes after exercise. Exercise may be prematurely terminated by exhaustion. In healthy individuals, plasma glucose rises or remains constant and may very rarely fall, but performance is unaffected. If measured,
the low plasma insulin concentrations often fall into the undetectable range.14 “Non-hypoglycaemic” patients become exhausted, but they have normal glucose and insulin responses to exercise, whereas patients with spontaneous hypoglycaemia become exhausted and their plasma glucose concentrations fall into the hypoglycaemic range. Plasma insulin, C peptide, and/or proinsulin remain inappropriately high in those with endogenous hyperinsulinaemia, but appropriately suppressed in those with hypoinsulinaemic hypoglycaemia.

**Prolonged fast**

The prolonged fast has long been advocated as the test of choice for investigating fasting hypoglycaemia.4 The 48 hour fast is a non-physiological test, and therefore should replace the previously recommended 72 hour fast.15 However, it is rarely required and is expensive in terms of hospital admission and close monitoring. It should be reserved for those patients in whom hypoglycaemia remains a strong possibility but who have not experienced a documented spontaneous or provoked hypoglycaemic episode.

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The fast must be conducted in hospital under medical supervision. During the fast, the patient is allowed to drink non-caloric and caffeine-free beverages. The patient must be encouraged to be ambulant during the hours and should be regularly tested for often subtle neuroglycopenia if plasma glucose approaches the hypoglycaemic range. Blood samples are collected every six hours until plasma glucose is 3.5 mmol/litre, when the sampling interval is reduced to every one to two hours. Blood samples are immediately analysed for plasma glucose and plasma or serum stored frozen for later measurement of pancreatic β cell products and β hydroxybutyrate. The fast is terminated, after adequate specimen collection, when plasma glucose falls below 2.5 mmol/litre and the patient has neuroglycopenic symptoms. In the absence of symptoms and hypoglycaemia, the test is terminated at 48 hours. The patient is fed at the end of the test. A few healthy individuals, usually young women, may have plasma glucose concentrations in the range of 2.5 mmol/litre or less following prolonged fasting, and may be misdiagnosed as having ketotic hypoinsulinaemic hypoglycaemia. However, they do not develop symptoms, emphasising the importance of testing clinically for neuroglycopenia.

**Mixed meal test**

The mixed meal test is used to investigate patients who experience postprandial neuroglycopenic symptoms for the possibility of reactive hypoglycaemia. There are no standard protocols, although it is recommended that the patient should consume a meal similar to the meal that led to symptoms during everyday life. Free flowing capillary blood samples are collected before and at 30 minute intervals for six hours after ingestion of the mixed meal. The test is considered positive if the patient develops neuroglycopenic symptoms in the presence of a capillary plasma glucose level of 3.0 mmol/litre or less.7 Venous blood should not be used because it may give false positive results—postprandial glucose concentrations in venous samples may be 1 to 2 mmol/litre lower than in the corresponding capillary samples.

The prolonged (five hour) 75 g glucose tolerance test is not recommended in the investigation of hypoglycaemia because it is a non-physiological test, and a large number of healthy subjects will have a false positive result, especially if venous blood samples are collected.15-17

**Sample and analytical considerations**

**Glucose**

Hypoglycaemia should be documented by laboratory glucose measurement. Glucose meters and especially visually read glucose test strips are unsuitable for the diagnosis of spontaneous hypoglycaemia in the domestic environment because many of the glucose methods used may be unreliable in the hypoglycaemic range and may mislabel healthy individuals as having hypoglycaemia.18-20 However, glucose meters may be useful in the clinical environment (such as in accident and emergency departments) as a rapid guide to the need for further blood collection (for confirmation and further investigation), immediately followed by the administration of glucose to relieve symptoms. Also of concern, but difficult to identify, is that the indiscriminate use of glucose meters may misclassify subjects with genuine spontaneous hypoglycaemia as being normoglycaemic.

Arterial blood glucose concentrations determine the development of neuroglycopenia. In the fasting state there is little difference between the glucose concentrations found in arterial and venous blood samples. As a result of the tissue uptake of glucose, postprandial venous blood glucose concentrations may be 1 to 2 mmol/litre lower than in the corresponding arterial samples, and may give rise to pseudohypoglycaemia. However, arterial blood sampling is impractical, but free flowing capillary blood is suitable because its glucose concentrations approximate very closely to arterial blood. In contrast, stagnant capillary blood results in serious underestimation of arterial glucose concentrations.

It is widely recognised that documentation of hypoglycaemia is important to prevent unnecessary and wasteful investigations and possibly erroneous diagnosis. Therefore, it is difficult to explain why 57% of samples received in a supraregional assay service laboratory for the investigation of hypoglycaemia were inappropriate, having glucose values of greater than 3.0 mmol/litre.

**Insulin, C peptide, and proinsulin**

With the development of widely available specific insulin assays, these have largely replaced non-specific insulin assays (also termed immunoreactive insulin or IRI), which measure not only insulin but also detect proinsulin and its partially processed fragments. Very specific insulin assays, unlike IRI assays, may fail to detect new synthetic insulins and insulinasmas exclusively secreting proinsulin.

C peptide is co-secreted with insulin from the pancreas in equimolar concentrations. The shorter half life and the hepatic extraction of insulin ensures that the molar concentration of C peptide in the peripheral circulation is several times higher than insulin. Its major clinical use is in the detection of exogenous insulin induced hypoglycaemia. C peptide is cleared by the kidneys, and is therefore raised in renal impairment. This may cause some difficulties in the investigation of hypoglycaemia in patients with renal disease.

Proinsulin normally represents less than 10% of circulating IRI. The greatest use of the proinsulin assay is in the diagnosis of an insulinoma secreting exclusively proinsulin.

Insulin, proinsulin, and C peptide immunoassays are potentially subject to interference from non-analyte antibody binding substances, including antibodies to insulin and to proinsulin. Therefore, it is important that laboratories investigating hypoglycaemia offer, as a minimum, the measurement of insulin, C peptide, and proinsulin because an inconsistency in the results could point to interference in an assay.

**Anti-insulin antibodies and insulin receptor specific antibodies**

Anti-insulin antibodies can be raised in response to exogenous insulins, but this is less common with the human insulins than with the previously administered animal insulins.
Anti-insulin autoantibodies also occur in patients never exposed to exogenous insulin, and may cause reactive hypoglycaemia in a syndrome described as autoimmune insulin syndrome (AIS). However, anti-insulin antibodies considered sine qua non for the diagnosis of AIS may also be present in non-hypoglycaemic individuals, and even rarely in patients with insulinoma.

Anti-insulin receptor antibodies (IR-A), depending on mode and site of action, may cause either hyperglycaemia as a result of insulin resistance or, very rarely, refractory hypoglycaemia. The diagnosis of IR-A mediated hypoglycaemia requires the demonstration of IR-A in the serum.

DIFFERENTIAL DIAGNOSIS OF CONFIRMED HYPOGLYCAEMIA

An algorithm for the differential diagnosis of documented hypoglycaemia is given in fig 1, which will elucidate most causes of hypoglycaemia.

Particular attention should be paid to drug history, especially in the presence of co-existent disease or exercise, or both. In the ill hospitalised patient, it is usually sufficient to recognise the underlying disease and its association with hypoglycaemia, without further investigation. However, confirmation of the underlying mechanism (see algorithm) may be sought.

SPECIAL ASPECTS OF HYPOGLYCAEMIA

Hyperinsulinaemic versus hypoinsulinaemic hypoglycaemia

Patients with hypoglycaemia can be classified into those with (inappropriate) hyperinsulinaemia or (appropriate) hypoinsulinaemia.

The hallmark of hyperinsulinaemic hypoglycaemia is inappropriate insulin secretion, not necessarily excessively high peripheral insulin concentrations, in the presence of hypoglycaemia. Although rare, the most common cause of endogenous hyperinsulinaemic hypoglycaemia is insulinoma, which is characterised by inappropriately high insulin and/or proinsulin, high C peptide, and suppressed low β-hydroxybutyrate.
(β-OHB) serum concentrations. It is noteworthy that a pure proinsulinoma may be missed if very specific insulin assays are used. In confirmed insulinomas, serum calcium should be measured because insulinomas may be a feature of type 1 multiple endocrine neoplasia. Selective pancreatic arterial calcium stimulation, endoscopic ultrasound, and intraoperative ultrasound may be of value in the localisation of the insulinoma; other imaging techniques are unreliable and may be misleading. Because almost all insulinomas are pancreatic, their successful localisation and removal depends on surgical experience and expertise.

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Other causes of hyperinsulinaemia, including factitious hypoglycaemia, autoimmune hypoglycaemia, and reactive hypoglycaemia should be excluded before making a diagnosis of insulinoma. This is especially important for factitious sulphonylurea induced and Regaplnide induced hypoglycaemia, which may produce an identical clinical and biochemical picture to insulinoma. Exclusion, by showing an absence of these compounds in blood and urine by a sensitive method at the time of hypoglycaemia, is essential to prevent unnecessary laparotomy. Hypoglycaemia resulting from exogenous insulin administration is easily distinguished from that caused by endogenous hyperinsulinaemia, in which case there will be inappropriately high insulin concentrations in the presence of low or suppressed C peptide values. Very rarely, this picture may be produced by insulinomas exclusively secreting proinsulin, if non-specific insulin assays are used, and by IR-A mediated hypoglycaemia. Proinsulinomas can readily be identified by the presence of absolute hyperproinsulinaemia. The diagnosis of IR-A mediated hypoglycaemia requires the demonstration of IR-A in the serum, and should be considered in patients with autoimmune disease and some varieties of neoplastic disease. It is also worth noting that some very specific insulin immunosassays fail to detect the recently introduced synthetic insulins and, therefore, may fail to identify their factitious or felonious use. The diagnosis of reactive hypoglycaemia is usually obvious on clinical grounds.

Other causes of hypoglycaemia (table 1) are associated with suppressed insulin concentrations and are termed hypoinsulinaemic hypoglycaemia.

**Ketotic versus non-ketotic hypoglycaemia**

Hypoglycaemia may also be classified as ketotic or non-ketotic. Low β-OHB (< 600 µmol/litre) during hypoglycaemia is indicative of increased insulin or insulin-like (insulin-like growth factor; IGF) activity and autoimmune hypoglycaemia, but can also occur in liver failure and in energy substrate deficiency (anorexia nervosa or starvation). All other causes of hypoglycaemia are associated with moderate to pronounced ketonaemia.

**Reactive (alimentary) hypoglycaemia**

Reactive hypoglycaemia occurs only in response to ingestion of a meal and generally occurs two to four hours after a meal. Reactive hypoglycaemia is relatively common after major gastric surgery and is sometimes termed the late dumping syndrome. It is otherwise rare, but may be a feature of the AIS, non-insulinoma pancreatic hypoglycaemia, or mild diabetes mellitus, or may be idiopathic or alcohol induced.

AIS should be considered in patients with autoimmune disease or previous exposure to sulphonylurea containing drugs, who present with hypoglycaemia and inappropriately high insulin and incompletely suppressed C peptide concentrations, with a disproportionately high insulin to C peptide molar ratio. In AIS, insulin released postprandially binds to the anti-insulin antibodies, resulting in hyperglycaemia, and as insulin is released from the insulin specific antibodies hypoglycaemia ensues. However, insulin specific antibodies, which are considered sine qua non for the diagnosis of AIS, may also be detected in non-hypoglycaemic individuals. Occasionally, the postprandial hypoglycaemia may be so delayed that the patient appears to have fasting hypoglycaemia.

Functional hyperinsulinism as a result of islet cell hyperfunction in NIPH can, as with insulinomas, be demonstrated by a twofold to threefold increase in hepatic venous insulin during a pancreatic artery calcium stimulation test. All causes of fasting hypoglycaemia may be associated, and very rarely present, with reactive hypoglycaemia. Therefore, idiopathic reactive hypoglycaemia may only be diagnosed with confidence after the exclusion of fasting hypoglycaemia.

**Non-islet cell tumour hypoglycaemia**

The term non-islet cell tumour hypoglycaemia (NICTH) is usually applied to hypoglycaemia caused by tumour that is not an insulinoma. NICTH is most frequently caused by excessive tumour secretion of abnormal IGF-II (big IGF-II), but also includes other very rare causes of hypoglycaemia, such as IR-A, insulin-binding monoclonal gammopathy, or tissue destruction by tumour causing major organ failure or endocrine disease.

The insulin-like activity of big IGF-II leads to hypoglycaemia, with consequent suppression of β cell secretion, lipolysis, and ketogenesis. Feedback of big IGF-II on the hypothalamic–pituitary axis suppresses growth hormone secretion, with subsequent lowering of growth hormone (GH) dependent IGF-I and IGF binding proteins secreted by the liver. Therefore, tumours secreting big IGF-II are characterised by an increased total IGF-II to IGF-I ratio, suppressed insulin and C peptide, and inappropriately low GH and β-OHB concentrations.

**FORENSIC ASPECTS OF DEATH FROM HYPOGLYCAEMIA**

Felonious (and factitious) hypoglycaemia suspected during life are relatively easy to confirm and investigate. Hypoglycaemia, however, is virtually impossible to diagnose after death. Blood collected after death from the right atrium, right ventricle, inferior vena cava, and hepatic vein gives misleading information because hepatic glycogenolysis, which begins immediately after death, leads to a substantial rise in blood glucose concentrations in these locations. Because the rate of glucose disappearance from extracellular fluid after death is unknown in humans, glucose analysis in peripheral blood, vitreous humour, and cerebrospinal fluid (CSF) can only be used to eliminate but not confirm hypoglycaemia as a factor in the death of an individual.

“Hypoglycaemia is virtually impossible to diagnose after death.”

In contrast, insulin, C peptide, and proinsulin may remain detectable for several days after death. In cases of unexpected death when hypoglycaemia is suspected, examination of the body for injection sites should be made and if found the needle tracts and surrounding tissue excised and examined immunohistologically for insulin. Samples should also be collected from a peripheral vein and artery, or possibly CSF. Serum should be separated and frozen until analysis. Whole blood should not be frozen because consequent haemolysis will invalidate subsequent analysis. Blood samples should not be collected from the heart or major blood vessels because they may be contaminated by the postmortem diffusion of insulin, C peptide, and proinsulin from the pancreas. High serum
insulin in the presence of low C peptide in patients without diabetes is indicative of exogenous insulin administration, although insulin will not be found if death results after prolonged coma. Sulphonylureas found in postmortem blood or urine samples in patients without diabetes suggests that hypoglycaemia may have contributed to death.

Insulin, C peptide, and sulfonylurea immunoassays are sufficiently robust for most clinical purposes, but they are subject to analytical error. Minimum immunoassay requirements for forensic purposes are to exclude non-specific interference by recovery and dilutional experiments, but it is possible that definitive assays using mass spectrometry may be required for forensic studies in the future.

SUMMARY

Hypoglycaemia may occur as an epiphenomenon in many serious diseases. It is sufficient to recognise the association of the disease with hypoglycaemia and then take appropriate action to prevent recurrence of hypoglycaemia.

In investigating apparently healthy individuals, common pitfalls to avoid are: failure to recognise subacute neuroglycopenia as a feature of spontaneous hypoglycaemia; failure to document hypoglycaemia adequately during symptoms; failure to measure pancreatic hormones, counter-regulatory hormones, and ketones in hypoglycaemic samples; failure to recognise limitations of laboratory assays; and failure to abandon obsolete investigations, such as the oral glucose tolerance test.

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