Amylase

What is it?

The amylases are a group of enzymes responsible for the breakdown of starches into sugars. The important amylase in animals is α-amylase (EC3.2.1.1 – alternate names 1,4-α-D-glucan glucanohydrolase, or glycogenase) which is a calcium metalloenzyme that acts at random locations along a starch molecule hydrolyzing α-1,4 links to ultimately yield mono-, di- and trisaccharides. Its primary function is as a digestive enzyme and its optimum operating pH is 6.7–7.0.

Plants, bacteria and fungi contain β-amylase which works along the starch polymer cutting off disaccharides from the reducing end. This is a much slower process than randomly splitting a large molecule into smaller parts because the number of substrate binding points remains constant (Moss and Henderson, 1999). The amylase gene has been duplicated during human evolution: this is thought to have had an important survival benefit. The number of gene copies is related to the saliva enzyme concentration. Thus, in the Japanese who have a diet traditionally high in rice starch, higher copy numbers are found than in the Biaka, rainforest dwellers with a low starch diet (Perry et al, 2007).

The primary sources of amylase in humans are the salivary gland and pancreatic juice. Salivary amylase (S-type amylase) and pancreatic amylase (P-type amylase) are distinct isoforms which means that although there are major similarities in primary protein structure, there are distinct differences which mean the two types of amylase can be distinguished analytically. However, in routine practice, it is usually sufficient to measure total amylase activity and to relate the assay result to the clinical picture.

How do we measure amylase?

Amylase activity is assayed by mixing a measured quantity of serum with a substrate reagent in a pH buffer and then measuring the subsequent change in concentration of the properties of the mixture (Moss and Henderson, 1999):

- Amyloclastic assays measure the decrease in concentration of starch, by using the starch-iodine colour reaction or by measuring turbidity
- Saccharogenic assays measure the increase in reducing sugar activity
- Chromogenic assays use dye-labelled substrates and measure the concentration of dye released.

Most assays in common use are now the defined-substrate chromogenic type.

S-type and P-type amylases can be measured individually by a variety of techniques. The different enzymes may be physically separated by electrophoresis, ion exchange chromatography or isoelectric focussing, or S-type amylase can be selectively inhibited by a protein isolated from wheat germ or a specific immunoglobulin. Finally, a specific immunoassay for P-type amylase has been developed (Moss and Henderson, 1999).

Why do we measure it?

The usual reason for measuring amylase is to assist in differential diagnosis of acute abdominal pain, as elevated amylase concentrations are consistent with inflammation of the pancreas. It is possible to measure other enzymes, specifically pancreatic lipase which is more specific for pancreatitis but the difficulty of maintaining a stable lipid substrate solution and of reading the light absorption changes of the reaction mixture mean that this is not commonly used in routine practice. Opinion varies whether lipase (Smith et al, 2005) or amylase (Ignjatović et al, 2000) is superior but practicalities mean that most laboratories prefer amylase to lipase.

Problems and pitfalls

Many articles have been written about the use of amylase for diagnosis of pancreatitis. Often, a set cut off is identified that is claimed to be 'diagnostic' (Matull et al, 2006). However, this is particularly problematical because there are a great many different assay methods in use for amylase. Indeed, more than 200 different assay methods have been described (Foo, 1995). Consequently, different hospitals will have different reference ranges for normality: for example, reference intervals for amylase range from 90–300 U/litre (Phadebas, Magle AB, Lund, Sweden) through 25–115 U/litre (Dimension RxL, Dade Behring, Milton Keynes, UK) to 22–89 U/litre (Olympus, Clare, Ireland) (Viljoen and Twomey, 2007). Thus, a 'diagnostic cut off' derived in one hospital may be utterly inappropriate in another, and similarly a cut off may become inappropriate as a result of changes in laboratory instrumentation. It is therefore important to know the reference range relating to the local laboratory, and not to try to remember the values that applied elsewhere.

As stated above, the usual reason for assaying amylase is differential diagnosis of acute abdominal pain. However, increases in amylase may occur as a result of other conditions. Of particular importance is an increase in total amylase as a result of S-type amylase which can be increased with diseases of the salivary glands, e.g. mumps, parotid tumours, trauma or obstruction (Howieson and MacKinlay, 2006). Acute abdomen with elevated amylase has been described in children, in association with parotid swelling (Howieson and MacKinlay, 2006). Given the rarity of non-traumatic pancreatitis in children it has been recommended that amylase is not used to investigate acute abdominal pain in paediatric patients (Wheeler et al, 1992).

Misattribution of salivary-derived amylase to abdominal pain could be solved by using a P-type amylase specific assay but, even then, there are several causes of increased pancreatic amylase (*Table 1*) that are not pancreatitis, including acute appendicitis, peritonitis, mesenteric ischaemia, bowel obstruction and biliary obstruction (Howieson and MacKinlay, 2006). Amylase has also been found to be elevated after burns and postoperatively.

Finally, amylase is cleared from the circulation by the kidney. Anything which interferes with this renal clearance will give rise to elevated serum amylase activity.

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Table 1. Causes of hyperamylasaemia and hyperamylasuria

P-type amylase↑	Pancreatic disease	Acute pancreatitis
		Complications
	Chronic pancreatitis	Pseudocyst
		Ascites
		Abscess
	Pancreatic trauma	
	Pancreatic carcinoma	
	Non-pancreatic intra-abdominal disease	Perforated peptic ulcer
		Intestinal obstruction
		Mesenteric infarction
		Acute appendicitis
	Drugs	Medicinal opiates
S-type amylase↑	Salivary gland disease	Mumps
	Salivary calculus	
	Macroamylasaemia (predominantly S-type)	
	Neoplastic hyperamylasaemia	Bronchogenic carcinoma (usually S-type)
		Ovarian carcinoma (usually S-type)
	Postoperative (usually S-type)	
	Renal transplantation	
	Non-pancreatic intra-abdominal disease	Ruptured ectopic pregnancy
	Drugs	Heroin addiction (?heroin lung)
Mixed	Renal insufficiency	
	Cerebral trauma (type depends on other injuries)	
	Burns and traumatic shock	
	Diabetic ketoacidosis	
	Acute alcoholism	
	Non-pancreatic intra-abdominal disease	Peritonitis (type depends on cause)
		Dissecting aortic aneurysm

From Moss and Henderson (1999)

Consequently it may be increased in renal failure and can be elevated as a result of complexation of amylase with immunoglobulin G or immunoglobulin A to form macroamylase (Lawson, 2001). Up to 5% of 'normal' subjects have been shown to have macroamylase demonstrable by polyethylene glycol (PEG) precipitation and therefore this must be considered in any patient where the enzyme results do not fit the clinical picture.

An alternative method of investigation in these patients is to measure serum and urine amylase. High levels of serum amylase would be expected to be associated with high urine amylase, but if the cause of the increased serum amylase is a macroamylase, this is not filtered by the kidney and therefore urine amylase will be low. Macroamylase can be transient or persistent and has been demonstrated to be associated with abdominal pain, pancreatitis, alcoholism, renal or liver disease, diabetes, cancer, inflammatory bowel disease or conditions of disordered immunity (Remaley and Wilding, 1989) including human immunodeficiency virus (HIV) infection (Foo and Konecny, 1997). BJHM

Conflict of interest: none.

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KEY POINTS

- The primary sources of amylase in humans are the salivary gland and pancreatic juice.
- The usual reason for measuring amylase is to assist in differential diagnosis of acute abdominal pain.
- Increases in serum amylase activity may occur as a result of other non-pancreatic conditions.
- Given the rarity of non-traumatic pancreatitis in children it has been recommended that amylase is not used to investigate acute abdominal pain in paediatric patients.
- Owing to the range of amylase methods, it is important to know the reference range relating to the local laboratory, and not to try to remember the values that applied elsewhere.