HbA$_{1c}$: Chasing numbers or considering context?

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Glycated haemoglobin (HbA$_{1c}$) reflects the average blood glucose level in the three months preceding the test. Changes in consecutive HbA$_{1c}$ tests indicate deteriorating, or improved, glycaemic control. HbA$_{1c}$ is considered to be the "gold standard" measure of blood glucose control and is often used as the basis for prescribing choices and other care decisions. A number of factors can affect the accuracy of the HbA$_{1c}$ result, for example, the life span of red blood cells, assay methods and clinicians' awareness of possible interfering factors. The aim of this article is to outline how HbA$_{1c}$ is used as a diagnostic test, how it is used to monitor glycaemic control and how it can guide management decisions. It is also important to emphasise the importance of considering HbA$_{1c}$ in the context of the individual rather than as an isolated number.

Glycated haemoglobin (HbA$_{1c}$) is an important guide to glycaemic control and influences treatment decisions. Several epidemiological studies, including the DCCT (Diabetes Control and Complications Trial; 1993) and the UKPDS (United Kingdom Prospective Diabetes Study; Turner et al, 1998) demonstrated that elevated HbA$_{1c}$ is a strong predictor of diabetes-related morbidity. Two consecutive HbA$_{1c}$ levels greater than 48 mmol/mol (6.5%) is associated with a linear increase in the incidence of diabetes complications, especially if the HbA$_{1c}$ values continue to increase (DCCT, 1993; Turner et al, 1998). Consequently, HbA$_{1c}$ is now an accepted measure of long-term glycaemia, as well as a clinically relevant measure of glycaemic control.

Although, there is an increasing body of evidence to suggest HbA$_{1c}$ alone does not always accurately reflect the individual's metabolic status (Weber and Schnell, 2009). Recent research suggests glucose variability (peaks and nadirs lasting minutes to hours during the day) is a risk factor for microalbuminuria (Hsu et al, 2012), adverse cardiovascular outcomes (Picconi et al, 2012) and all-cause mortality in type 2 diabetes (Ma et al, 2011).

Glycaemia and diabetes management

HbA$_{1c}$ was measured chromatographically in the DCCT and UKPDS. Chromographic methods detect several haemoglobin variants and non-HbA$_{1c}$ glycohaemoglobins and tend to overestimate HbA$_{1c}$ compared to modern assay methods (Larese, 2012). A considerable international effort has been undertaken in recent years to standardise existing HbA$_{1c}$ assays in current use and to standardise how glycated haemoglobin and estimated average blood glucose are reported (National Glycohemoglobin Standardisation Program, www.ngsp.org).

HbA$_{1c}$ in people without diabetes usually ranges between 20 and 41 mmol/mol (4–5.9%; Diabetes.co.uk, 2013). HbA$_{1c}$ consistently >53 mmol/mol (7%) in people with diabetes

Article points

1. Glycated haemoglobin (HbA$_{1c}$) is an important guide to glycaemic control and influences treatment decisions. Two consecutive HbA$_{1c}$ levels greater than 48 mmol/mol (6.5%) is associated with a linear increase in the incidence of diabetes complications, especially if the HbA$_{1c}$ continues to increase.

2. A number of factors can affect HbA$_{1c}$, for example, conditions that affect red blood cell survival and non-enzymatic glycosylation of haemoglobin. Other factors include genetics, female gender, sex hormones, visceral fat distribution and the HbA$_{1c}$ assay method.

3. It is important to consider HbA$_{1c}$ results in the context of the individual when making diagnostic and therapeutic decisions.

Key words

- Haemoglobinopathies
- HbA$_{1c}$
- Hyperglycaemia
- Hypoglycaemia

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represents inadequate glycaemic control and is associated with increased risk of long-term diabetes complications (Ray et al, 2009). The American Diabetes Association (ADA, 2011) and the World Health Organisation (WHO, 2011) recommend aiming for an HbA$_{1c}$ value <48 mmol/mol (6.5%).

NICE (2009) guidelines for type 2 diabetes recommend aiming for HbA$_{1c}$ levels <48 mmol/mol (6.5%) for people taking one blood glucose-lowering medicine (GLM) and <58 mmol/mol (7.5%) for those taking two or more GLMs. However, NICE acknowledged these levels may not be appropriate for all people with type 2 diabetes. Likewise, the Australian Diabetes Society (ADS, 2009) suggested a general HbA$_{1c}$ target of <53 mmol/mol (7%), but indicated some people should aim for <42 mmol/mol (6%) and some <64 mmol/mol (8%), depending on individual circumstances.

Significantly, HbA$_{1c}$ <53 mmol/mol (7%) is associated with more frequent hypoglycaemia, which puts some people with diabetes, particularly older people, at increased risk of adverse events, including cognitive impairment, impaired decision-making, falls and myocardial infarction (The ACCORD Study Group, 2011). Thus, there is growing recognition that HbA$_{1c}$ levels <53 mmol/mol (7%) may not be appropriate for everybody with diabetes and that management goals need to be individualised, especially in children and older people (ADS, 2009; NICE, 2009; Gale, 2010; Scottish Intercollegiate Guideline Network, 2010).

ADA (2011) have indicated that HbA$_{1c}$ values of 37–48 mmol/mol (5.7–6.4%) represent pre-diabetes, which is part of a continuum of diabetes risk and a marker of cardiovascular disease, and identifying pre-diabetes enables early treatment to be implemented to reduce the risk. Some authorities suggest repeating the test in asymptomatic people (d'Emden et al, 2012), which could mean diabetes is diagnosed earlier and treatment can be initiated sooner. Likewise, the WHO (2011) cautioned that an HbA$_{1c}$ <48 mmol/mol (6.5%) does not exclude diabetes. In addition, it is important to consider factors that cause false high or low HbA$_{1c}$ when interpreting results before making a definitive diagnosis.

HbA$_{1c}$ as a diagnostic tool

The WHO (2011), International Diabetes Federation (IDF; 2013) and the ADA (2011) recommend that HbA$_{1c}$ is used as a diagnostic test for diabetes and cited values of HbA$_{1c}$ 48 mmol/mol (6.5%) as the diagnostic cut-off point. HbA$_{1c}$ overcomes some of the methodological, procedural and practical problems associated with using blood glucose to make the diagnosis. These include the significant day-to-day variations in blood glucose levels, the fall in blood glucose concentration in blood samples, even when they are collected in fluoride-oxalate tubes, and inter-laboratory variations (d'Emden et al, 2012).

In Australia, d'Emden et al (2012) have stated that HbA$_{1c}$ has an important place in diagnosis when using the recommended diagnostic cut-off point, but suggested the test should be repeated in asymptomatic people with a diagnostic HbA$_{1c}$ result to confirm the diagnosis. They further stated that HbA$_{1c}$ should not be used as a general diabetes screening test and that initial screening should still be based on the Australian Type 2 Diabetes Risk Assessment Tool (AUSDRISK) score (Australian Government Department of Health and Ageing, 2008). The AUSDRISK is widely used in Australia to screen for diabetes; other countries have similar screening tools, such as the UK Diabetes Risk Score (Diabetes UK, 2013). In addition, d'Emden et al recommend that health professionals consider other factors that can affect the HbA$_{1c}$ level when interpreting the result and making management decisions; see *Box 1* and *Table 1*.

Box 1. Main types of haemoglobinopathies

- Sickle cell anaemia.
- Sickle cell haemoglobin A disease.
- Sickle cell beta thalassaemia.
- Sickle cell haemoglobin D disease.
- Thalassaemia beta variants.
- Other rare genetic variants.

Table 1. Factors that affect HbA$_{1c}$ (adapted from Herman, 2009; Nithin, 2010; WHO, 2011; Larese, 2012).

Factors that can increase HbA$_{1c}$	Factors that can lower HbA$_{1c}$
Asplenia	Recent blood transfusion
Genetic heterozygous variants of haemoglobins S, C and E	Increased erythropoiesis secondary to haemolysis or blood loss
Large vitamin C doses with some assay methods*	Acute and chronic blood loss
Long-standing iron deficiency	Haemolytic anaemia
B$_{12}$ deficiency	Hereditary persistence of haemoglobin F
Medications, such as corticosteroids and antipsychotic agents	Genetic heterozygous variants of haemoglobins S, C and E*
Malaria	End-stage kidney disease due to anaemia, malnutrition and haemodialysis
Rheumatoid arthritis	Large vitamin doses of vitamin C and E
Hyperbilirubinaemia	Recent iron infusion
High triglycerides	Recent B$_{12}$ injection
Third trimester of pregnancy†	Erythropoietin
	Chronic alcohol, opioid and/or high doses of salicylates
	Second trimester of pregnancy†

*Especially in African-Caribbean people
†However, changes are small (<1%)

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HbA$_{1c}$ is less reliable as a diagnostic tool in certain groups people, for example, those with cystic fibrosis-related diabetes (Dyce and Wallymahmed, 2012); people on long-term corticosteroid medicines (Tidy, 2012); those with long-standing iron deficiency anaemia (Stranks and Doogue, 2012) and people with renal disease on haemodialysis (Vos et al, 2011). In addition, point-of-care HbA$_{1c}$ test methods are useful to monitor glycaemic control but may not be accurate enough to diagnose diabetes (International Expert Committee, 2009).

Factors that affect HbA$_{1c}$

A number of factors can affect HbA$_{1c}$, especially conditions that affect red blood cell survival and non-enzymatic glycosylation of haemoglobin (Herman, 2009; d'Emden et al, 2012). Herman (2009) suggested only approximately one third of the variance in HbA$_{1c}$ is due to glycaemia. Other factors that affect HbA$_{1c}$ include genetics, female gender, sex hormones, visceral fat distribution (Cohen et al, 2006) and the HbA$_{1c}$ assay method. For example, immunoassay methods yield lower results than high performance liquid chromatography (HPLC; Schweitzer et al, 2012).

Formation of glycated haemoglobin

Glycation is a non-enzymatic, time-dependent chemical process during which glucose binds to the amino proteins in various tissues in the body (Little and Roberts, 2009). Glucose attaches to haemoglobin in the blood and several processes occur to form HbA$_{1c}$. Several HbA peaks can be observed on chromographic assays: HbA$_{1a}$, HbA$_{1b}$ and HbA$_{1c}$. HbA comprises 96–98% of the total haemoglobin and HbA$_{1c}$ is the dominant component that

correlates with the mean serum glucose. Thus, HbA$_{1c}$ specifically refers to glycohaemoglobin where glucose binds to the terminal valine of the beta-haemoglobin chain to form an adimine link, which undergoes an Amadori reaction and forms a stable ketoamine link.

Glycosylated and non-glycosylated hexapeptides are separated using HPLC and quantified. HbA$_{1c}$ is reported as the ratio of its concentration to total HbA (Jeppsson et al, 2002) and represents the average blood glucose over the preceding 2–3 months (Nitin, 2010). Fasting plasma glucose (FPG) and post-prandial glucose (PPG) have the same effect on HbA$_{1c}$ <53–64 mmol/mol (7.3–8.4%). However, FPG has a greater effect when HbA$_{1c}$ is >63 mmol/mol (8.5%); the higher the HbA$_{1c}$, the more FPG contributes to total blood glucose.

HbA$_{1c}$ assays

Several assay methods are used to measure HbA$_{1c}$ including iron exchange, HPLC, chemiluminiscent immunoassays, boronate affinity and enzymatic methods (Herman 2009; Nitin 2010). The US-based National Glycohemoglobin Standardisation Program was established in 1996 to standardise the various methods to the DCCT trial equivalent methods (Herman, 2009). Each year the College of American Pathologists compares within and between HbA$_{1c}$ values and publishes the findings. These initiatives have improved comparability among the various methods. However, as indicated, a number of factors, independent of the method, can affect the results (*Box 1* and *Table 1*) and need to be considered when interpreting the individual's HbA$_{1c}$ results.

Considering the individual

The preceding information raises several important points that support the need to interpret the HbA$_{1c}$ results in the context of the individual, including whether the HbA$_{1c}$ value is diagnostic of diabetes, warrants a management change, or represents a complication risk. HbA$_{1c}$ does not always reflect the individual's blood glucose self-monitoring pattern for a number of reasons, including inaccurate testing

technique. HbA$_{1c}$ is unreliable when erythrocyte abnormalities are present (d'Emden et al, 2012) and in the presence of haemoglobinopathies (Weber and Schnell, 2009) and anaemia. Weber and Schnell (2009) reported that seven out of 29 people (24%) with lower than expected HbA$_{1c}$ based on their home blood glucose test results had a haemoglobinopathy detected on electrophoreses.

Haemoglobinopathies are inherited abnormalities of haemoglobin and are often asymptomatic. Haemoglobinopathies are one of the most common factors affecting HbA$_{1c}$ levels worldwide, depending on the assay used to measure HbA$_{1c}$. Most HbA$_{1c}$ assays assume the individual has a normal erythrocyte lifespan and that the assay method is specific for non-enzymatic adduction of glucose to haemoglobin, which may not be the case if the individual has a haemoglobinopathy. There are several haemoglobinopathies, including thalassaemia and many other Hb variants (see *Box 1*). Haemoglobinopathies commonly occur in people from Africa, the Mediterranean and south-east Asia and are uncommon in Caucasian people. Research has shown that the difference in HbA$_{1c}$ between Black people and Caucasian people becomes greater as the blood glucose increases, and is higher in Black people than Causcasian people at the same blood glucose level (Ziemer et al, 2010).

Many countries have multicultural populations and intermarriage is becoming increasingly common; therefore, health professionals need to consider haemoglobinopathies and other factors that affect HbA$_{1c}$ when interpreting results. It is possible to screen for haemoglobinopathies but few countries have haemoglobinopathy screening programmes in place. The British Society for Haematology developed guidelines for antenatal and newborn haemoglobinopathy screening, which recommend selectively screening high-risk women and that universal screening might be warranted in areas with a high proportion of at-risk individuals (Ryan et al, 2010). It is not clear whether these recommendations are in routine use. Screening for haemoglobinipathies does not appear to be

established in other countries.

HbA$_{1c}$ can be spuriously elevated or lowered by a number of interfering factors besides haemoglobinopathies. For example, factors that reduce the lifespan of red blood cells lead to low HbA$_{1c}$. Factors that lead to increased HbA$_{1c}$ include infections and medicines such as corticosteroids and antipsychotics. *Table 1* depicts some factors known to reduce or increase HbA$_{1c}$.

Person-centred care is an important philosophy underlying modern diabetes education and management, and this should be applied to interpreting HbA$_{1c}$. HbA$_{1c}$ is part of an individual's clinical picture and needs to be considered in context because it does not portray the whole picture.

Factors that affect the HbA$_{1c}$ need to be identified and managed. In addition, it is important to:

● Involve the individual and any family members or carers in decisions about investigative procedures and care.

● Listen to the individual with diabetes and ask appropriate questions to identify factors that could influence education and management decisions.

● Understand haemoglobinopathies are rare in Caucasian people, but do occur, especially in multicultural societies. Consider whether individuals from at-risk groups should be screened for a haemoglobinopathy.

● Realise very few countries have effective screening programs to detect haemoglobinopathies before or at birth. Thus, the true prevalence may be unknown in specific countries and populations.

● Consider common and uncommon factors that increase or lower HbA$_{1c}$ when deciding an individual's diabetes management regimen.

● Consider using fructosamine to monitor glycaemic status as an alternative to HbA$_{1c}$ in individuals where HbA$_{1c}$ is unreliable. Fructosamine assays have been available since the 1980s but are not widely used in most countries. Fructosamine is formed in a non-enzymatic process where protein, especially serum albumin, is glycosylated. Fructosamine reflects the average blood glucose over the preceding 14–21 days; therefore, it can be useful to monitor short-term management changes, especially during pregnancy. There is no standard reference range for fructosamine; generally, the higher the fructosamine level, the higher the blood glucose. Like other targets, the fructosamine target should be individualised in collaboration with the individual. Any condition that affects serum albumin, such as nephritic syndrome or malnutrition, can interfere with the fructosamine result. Thus, the individual's health status and context must be considered when interpreting fructosamine results.

● Know the factors that lower HbA$_{1c}$ could impact on its utility as a diagnostic tool and that diagnoses could be missed or delayed and place the individual at risk of diabetes complications.

● Include prompts on pathology reports to alert health professionals to the need to check the individual's blood glucose pattern and consider the possibility of red blood cell abnormalities and other HbA$_{1c}$-related interfering factors if the HbA$_{1c}$ is repeatedly 42 mmol/mol (6%). Alerts are often used in this way to signal high HbA$_{1c}$. However, clinicians might only refer to the results and not consider alert messages in busy clinical settings.

Managing diabetes is complicated for both health professionals and people with diabetes. It is important to individualise care and respect the person's accumulated life and diabetes expertise. A number of factors beside blood glucose affect HbA$_{1c}$ levels and need to be considered when making therapeutic and diagnostic decisions. Clearly, HbA$_{1c}$ (and fructosamine, if it is used) is only part of the individual's diabetes story and should be considered with blood glucose self-monitoring tests and other important information. ■

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Page points

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