Glycosylated haemoglobin: The spy in the cab

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Today's diabetes world is fast-moving and exciting; knowledge is accumulating at an astonishing rate. To help understand the present, however, it sometimes helps to examine the past.

In this installment of *Tattersall's Tales*, Robert Tattersall returns to the days before the Diabetes Control and Complications Trial, before evidence to support the importance of glycaemic control, back to the discovery of glycosylated haemoglobin.

Now that a new way of reporting the results of haemoglobin A_{1c} (HbA_{1c}) assays has been introduced it is opportune to reflect on the contribution of the discovery of glycosylated haemoglobin in the history of diabetes (Collier et al, 2009).

People who came into diabetes after publication of the results of the Diabetes Control and Complications Trial (DCCT) in 1993 may find it difficult to imagine a time when the goals of treatment were uncertain. However, in the 1970s and 1980s, many physicians, especially in the USA, denied any connection between glucose control and microvascular complications.

The only evidence (as opposed to opinion) for or against the importance of glucose control was hundreds of retrospective clinical studies in which patients, usually from a single clinic, were ranked by the degree of control thought to have been achieved over a number of years. Control was assessed from three or four random blood sugars taken in the clinic each year (usually at the same time of day), episodes of ketoacidosis and the results of home urine tests. Surprisingly, in some studies, episodes of hypoglycaemia counted towards bad control.

Uncovering the evidence

In 1970, Harvey Knowles (1915–1984) of Cincinnati reviewed 300 retrospective studies and found only 85 that did not contain major errors of design – for example, in many, control and complications were assessed by the same person, which must inevitably introduce bias. Of the acceptable studies, 50 concluded that poor control caused microvascular disease, 25 found no relationship and 10 were undecided (Knowles, 1970).

The only way to solve the problem was a randomised controlled trial, which was impossible without an objective measure of glucose control. This came from an unlikely source. An Iranian physician, Samuel Rahbar (b.1929), worked in Cambridge with Hermann Lehmann (1910–1985), the expert on abnormal haemoglobins, who persuaded him to return to Iran and establish a haemoglobin research unit.

In 1967 Rahbar noticed that blood samples from some hospital patients had a fast-moving haemoglobin fraction, the concentration of which varied between 7% and 15% of total haemoglobin. This was odd because haemoglobin variants with alpha-chain abnormality constitute 25% of total hemoglobin and those with a beta-chain abnormality (e.g. HbS) constitute 40%. He investigated the relatives of the original patient but none had the same band, suggesting that this was not an inherited abnormality. Soon two other samples turned up with the same fast-moving band, and when Rahbar went to the ward where one of them was, he saw that "Diabetic" was written on her chart. He then studied samples from 47 patients with type 2 diabetes who all had the abnormal band but in differing concentrations. In 1968, he published a short report on a "new haemoglobin" in the blood of two people with diabetes (Rahbar, 1968).

This was not the first report of this "diabetic" haemoglobin. In 1962, Huisman and Dozy had reported an increase in a minor fraction of haemoglobin in four people with diabetes but thought it was an effect of tolbutamide (Huisman and Dozy, 1962).

In 1968 Rahbar went as a post-doctoral fellow to the Albert Einstein College of Medicine in New York where he worked with another haemoglobin expert, Helen Ranney (b.1920). They identified the "diabetic" haemoglobin as HbA_{1c}, which had been discovered 10 years earlier in the human fetus (Rahbar et al, 1969).

In 1973 when I was working on identical twins with diabetes, my boss David Pyke made contact with Helen Ranney and suggested that we send her samples from twins, especially those discordant for diabetes. David was always on the lookout for genetic markers of diabetes and thought that HbA_{1c} might be one. I duly collected and sent off the samples. Levels of HbA_{1c} were high in twins with diabetes and similar to those in controls without diabetes.

By the time the results came back, I was thinking about other things and did not grasp the significance of the results. We (I was the lead author) concluded that elevation of HbA_{1c} "is dependent on the disordered carbohydrate metabolism, and is not an independent component or genetic marker of the diabetic syndrome". Some twins with diabetes in the study were on diet alone and had normal levels of HbA_{1c}, which we suggested might reflect better control of carbohydrate metabolism (Tattersall et al, 1975). In 1976, this was shown to be the case by Koenig and co-workers, who found that when patients on insulin were brought under control in hospital, their HbA_{1c} concentrations returned to normal in 4–6 weeks (Koenig et al, 1976).

Spy in the cab

Rather than a genetic marker, HbA_{1c} was an independent monitor of glycaemic control. It gave clinicians a way of checking the control of their patients in the same way that the tachometer allowed truck operators to find out how many hours their drivers were working – hence the "spy in the cab" analogy.

Haemoglobin was the first example of a protein that was glycosylated in vivo, and it seemed likely that advanced glycation end-products (AGEs) might be the basis of diabetic complications and ageing. Hence it was hoped that blocking glycation would not only prevent diabetic complications but result in the Holy Grail – an anti-ageing drug. Vitamin E and aminoguanidine block production of AGEs in diabetic rats in the laboratory, but only modestly or not at all in human beings. Nevertheless, both are advertised on the internet as anti-ageing drugs.

In the early days HbA_{1c} was measured by column chromatography, which was susceptible to interference by other abnormal haemoglobins. I remember two cases particularly well: In the first I was doing a general medical clinic when an obstetric senior registrar rang in a panic about a Caucasian woman who was 6 months pregnant with an HbA_{1c} of 11.5% (102 mmol/mol). I asked what her blood sugar was and was told, "that's the odd thing, it is only 3.9 mmol/L". To calm the senior registrar I saw her within the hour and asked if she had any foreign ancestry to which she replied, "How did you know? My father is Icelandic". In fact this was not relevant since she turned out to have persistent fetal haemoglobin (HbF).

The second patient was a 30-year-old woman who had been diagnosed with type 1 diabetes at age 17. She was on twice-daily isophane and attended the clinic twice a year but never saw the boss because she never had any problems. However, one day she strayed into my orbit and I was struck by the discrepancy between her clinic blood sugars and HbA_{1c}. The former were all in single figures for the previous eight visits while the HbA_{1c} was always over 10% (86 mmol/mol). Her notes contained a series of letters from my registrars chiding her for her poor control and suggesting an increase in her insulin doses. At first she had dutifully complied but found that it resulted in disabling hypoglycaemia and decided to become "non-compliant", which one of my colleagues used to call the patient's defence against dangerous doctors! She also had persistent HbF.

Standardising the gold standard

Apart from interference from abnormal haemoglobins it was also (fairly) well known to clinicians that HbA_{1c} results could be misleading when there was abnormal red cell turnover. However, the main problem in the 1990s was the lack of standardisation between laboratories, which caused much confusion for patients and doctors. Hopefully this will have been resolved by the introduction of IFCC (International Federation of Clinical Chemistry and Laboratory Medicine) mmol/mol units, although the new numbers will take some getting used to.

Those who lived through the conversion to SI units in the 1970s will remember the problems that the Americans found so insurmountable that they soon reverted to the old units.

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