Diabetologia (2006) 49: 7–10
DOI 10.1007/s00125-005-0073-7

COMMENTARY

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How should HbA_1c measurements be reported?

Published online: 24 December 2005

Historical and biochemical background

In 1968, amidst the turmoil of student protests, a more discreet event—the reporting of increased minor Hb fractions in erythrocyte lysates from diabetic patients [1]—set the stage for yet another revolution, this time in our understanding of the pathogenesis of chronic complications of hyperglycaemia and in the management of diabetic patients. Since then, non-enzymatic glycation of proteins has been identified both as the reaction responsible for this increase in minor Hb bands [2], and as the first step in one of the key biochemical mechanisms underlying the development of chronic complications of diabetes [3]. Incidentally, the reaction of reducing sugars with amino groups was already long known to the food industry, when it was first identified, to the benefit of all of us, as the starting point of a complex series of reactions implicated in the formation of flavours and colours in, for example, beer, confectionery products and roasts (Fig. 1) [4].

Glycated Hb levels provide a time-integrated measure of glycaemia over the lifespan of the red blood cell (about 120 days), and therefore represent an index of glycaemic control for that period. They are used to assess the risk of developing chronic complications of diabetes and the quality of diabetes care [5]. It became progressively clear that non-enzymatic glycation reactions can occur to a significant degree between any free amino group on a protein (N-terminal or intracatenar groups from dibasic amino acids) on the one hand, and a free aldehyde or keto group from a variety of (phospho)hexoses or pentoses on the other, provided that the half-life of the protein is sufficiently long (Fig. 2) [5]. Glucose, as it happens, appears to be the least reactive aldohexose in this respect. Some have speculated that this property may have conferred a selective advantage to glucose, and that it was for this reason that it emerged as the universal metabolic fuel rather than one of the other hexoses or pentoses involved in carbohydrate metabolism [6]. Glycation apart, long-lived proteins such as Hb may undergo various other non-enzymatic post-translational modifications, such as carbamylation by urea or acetylation by acetylsalicylic acid.

Abbreviations ADA: American Diabetes Association · EASD: European Association for the Study of Diabetes · IDF: International Diabetes Federation · IFCC: International Federation of Clinical Chemistry · NGSP: National Glycohaemoglobin Standardization Program

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Why harmonise reporting of glycated Hb?

Over the past decades a wide range of assays based on a variety of analytical principles have been developed, each recognising various molecular species of (non-)glycated Hb—including genetic variants—to a variable degree, and each with their own reference range and differing susceptibility to interfering substances, such as reversibly Hb-bound glucose, foetal Hb or carbamylated Hb [5]. This created much confusion among health care providers, who were confronted with the indiscriminate use of terms such as (total) glycoHb, glycosylated Hb, HbA_1 or HbA_1c, and with results that were often difficult to compare and to translate into glycaemic control [5]. From this point in the article onwards, we will only use the term HbA_1c (referring to the stable adduct of glucose to the N-terminal valine of the β chain of Hb).

As outlined in a recent report of an American Diabetes Association/European Association for the Study of Diabetes/International Diabetes Federation (ADA/EASD/IDF) working group established in 2004 to harmonise HbA_1c reporting, the situation has progressively improved, thanks

Fig. 1 Non-enzymatic glycation and browning reactions in the kitchen: a culinary blessing

to a number of major collaborative efforts [7]. The DCCT and, later, the UK Prospective Diabetes Study (UKPDS) have provided a direct correlation between HbA_1c levels—traceable to a reproducible BioRex-70-based HPLC method (Bio-Rad Laboratories, Hercules, CA, USA) [7]—and clinical outcome measures in type 1 and type 2 diabetes respectively [8, 9]. The American National Glycohaemoglobin Standardization Program (NGSP) has, since then, strikingly improved between-laboratory comparability, resulting in near-complete reporting of HbA_1c as DCCT equivalents in the USA [10]. Similar harmonisation efforts have been made in Sweden and Japan with other anchor methods, yielding, however, lower HbA_1c values than those obtained in the NGSP [11].

The chromatographic 'HbA_1c' peak measured by the DCCT anchor method has been shown to contain various glycated and non-glycated Hb molecules [11]. The Japanese and Swedish anchor methods are also not absolutely specific, but their HbA_1c peaks are less contaminated by other substances [11]. Taken together, no single standardisation method fulfils all the requirements for the establishment of an international reference system. The International Federation of Clinical Chemistry (IFCC) therefore set out to develop a robust reference system by providing: (1) a clear definition of the analyte based on its molecular structure (see above); (2) a primary reference material in a pure form, namely, glycated (for HbA_1c) and non-glycated (for HbA_0) N-terminal hexapeptides from the Hb β-chain; (3) a validated reference method based on liquid chromatography-mass spectrometry that specifically measures the analyte in human samples; and (4) a global network of reference laboratories [12–14]. This IFCC reference method generates consistently lower HbA_1c results than the standardisation schemes in the USA, Japan and Sweden, but a firm and reproducible link between these four methods has been established [11]. It should also be made clear that, at present, this sophisticated reference method is not suitable for large-scale application in daily routine.

How can global harmonisation be achieved?

Possible options and immediate actions

The ADA/EASD/IDF Working Group of the HbA_1c Assay has recently proposed a road map for the achievement of global harmonisation of HbA_1c results [7, 15]. It considered three possible ways of dissipating residual international differences: (1) to change the assigned values of standards in existing commercial assays so that more accurate but lower IFCC-like HbA_1c results are reported; (2) to report outcomes-based DCCT-like HbA_1c results; (3) to report HbA_1c as mean blood glucose equivalents after having gained further insight into the relationship between the two parameters. Regardless of the final choice, there is consensus that the robust and specific IFCC method should be used as a global reference point for calibration of commercial HbA_1c methods [7, 15]. However results are reported, harmonisation should be achieved in a concerted and synchronised fashion, and not, as wisely advised by the working group, be driven by unilateral initiatives from manufacturers. The damage that such uncontrolled changes may produce was recently painfully exemplified when, following an EC directive, some manufacturers altered the standardisation and reference ranges of their creatinine kits to report 'true' serum creatinine values without consulting health care providers [16]. The inappropriate use of these more accurate creatinine results in algorithms that were designed long ago to estimate creatinine clearance on the basis of a serum creatinine level obtained with older and less specific methods caused considerable confusion and could lead to overestimation of creatinine clearance and er-

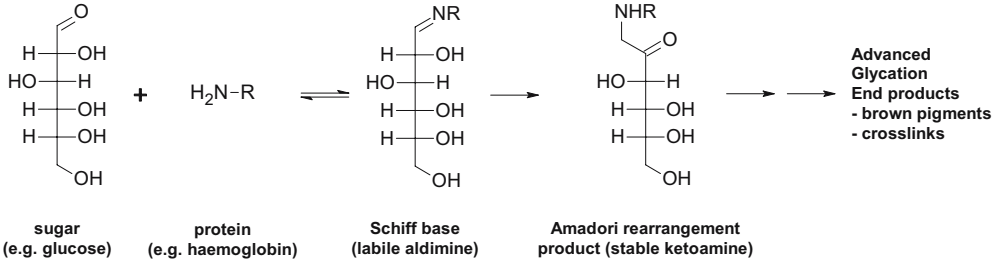

Fig. 2 Non-enzymatic glycation of proteins: initial reaction steps

rors in the administration of potentially toxic drugs [16]. It was thus very prudent of the ADA/EASD/IDF Working Group of the HbA_1c Assay to advise manufacturers not to change their way of reporting HbA_1c values until further information on the relationship between mean blood glucose and HbA_1c levels has been compiled. In the interim, continued use of DCCT/UKPDS equivalents has been advocated [7, 15].

New studies

Prospective studies designed to elucidate the relationship between mean blood glucose levels and HbA_1c have been launched in parallel, and should be completed in 2006/2007 [7, 15]. Although an overall significant relationship was retrospectively established in DCCT, the correlation was weak and the error limits wide [17]. For example, HbA_1c values ranged from 6.0–9.0% at a mean glucose concentration of 10 mmol/l [17, 18]. The imprecise relationship between glycaemia and HbA_1c is, in part, determined by the analytical uncertainty of the methods used [18]. Such comparisons thus require the use of HbA_1c methods with optimal precision and calibration frequency such that interassay CVs of <3% are achieved [19, 20]. Intra-individual and interindividual biological variability further contribute to the weak correlation between mean blood glucose and HbA_1c [18]. It should be appreciated that HbA_1c represents a weighted average of blood glucose over the preceding 120 days, whereby the most recent 30 days contribute about 50% [18]. Moreover, interindividual (genetic or acquired) differences in erythrocytic lifespan or (de)glycation rate have been reported and may cause variations in HbA_1c levels that are unrelated to mean blood glucose levels [18, 21, 22]. Furthermore, there are conflicting results as to whether premeal and postmeal glycaemia contribute equally to HbA_1c [18, 21]. The predominant view is that, both in patients with type 1 and type 2 diabetes, fasting glucose levels correlate more closely with HbA_1c than do postprandial values. It is thus conceivable that different blood glucose profiles may produce similar HbA_1c levels and vice versa. Hence, the statistical relationship between HbA_1c and glycaemia is, at present, difficult to apply at an individual level. Further prospective data are clearly needed to determine whether the relationship between mean blood glucose and HbA_1c might differ in various ethnic groups, especially in subjects with type 2 diabetes, or with extreme and rapidly changing values of mean blood glucose, during pregnancy, or in response to certain medications [7, 15].

The final choice

Reporting HbA_1c as 'true' IFCC values carries the disadvantage that the whole world would have to adapt to lower HbA_1c values, a situation that has been shown to be associated with an increased risk of metabolic deterioration in patients [23]. This risk should, in our view, not be allowed in a world where the majority of patients already do not reach the recommended treatment goals [24]. Reporting HbA_1c in familiar blood glucose units has the advantage that the meaning of the results can be better grasped by the patients, especially those that are used to home glucose monitoring. In addition, the glucose scale does not suffer from a number of inconveniences linked to the classical reporting of HbA_1c results, where patients may perceive small changes—although linked to large health effects—as unimportant.

The jury is still out as to the global applicability of the relationship between HbA_1c levels and mean blood glucose. If the ongoing studies identify practical problems in implementing this strategy, alternatives should be considered. In this respect, an obvious possibility would be to attempt global harmonisation of HbA_1c results by advocating the use of precise HbA_1c methods calibrated against the robust IFCC reference method and reporting results as DCCT-like equivalents by taking advantage of the firmly established relationship between the IFCC and DCCT methods. This would only require an adjustment of the HbA_1c scale and reference ranges in some parts of the world. Since there would be a shift to higher values in these countries, the risk of inducing metabolic deterioration should be low [23]. Last, but not least, the direct link with outcome measures from the DCCT and UKPDS would be preserved.

In the meantime, the IFCC has not officially endorsed the recommendations of the ADA/EASD/IFCC Working Group of the HbA_1c Assay and is still exploring other avenues, including reporting HbA_1c results directly as a percentage of total Hb and expressed as IFCC equivalents (hence yielding lower values and a lower reference range than in the DCCT), or, alternatively, switching to another name—deoxyfructosyl Hb (a chemical description of HbA_{1c})—and new units (mmol HbA_1c per mol total Hb) [25, 26]. The advantages of the latter approach include large numerical changes in the clinically relevant range and accuracy in chemical terms, but this would require substantial re-education efforts and produce added confusion for patients.

Conclusion

Everybody agrees that global harmonisation of HbA_1c reporting is important and necessary. However, at present, there is no consensus on what would finally be the optimum global means of expressing HbA_1c. The ADA/EASD/IDF Working Group of the HbA_1c Assay has defined a methodology to reach that goal in the coming years. In the meantime, it is of the utmost importance that no unilateral shortcuts are taken by manufacturers or the IFCC that would jeopardise the decision process outlined by health care providers.

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