Implications of using HbA_{1c} as a diagnostic marker for diabetes

Nadia Hussain¹

Received: 10 July 2015 / Accepted: 9 October 2015 / Published online: 6 November 2015
© The Japan Diabetes Society 2015

Abstract Diagnosing diabetes now includes a new criterion; hemoglobin A_{1c} ≥ 6.5 % which can have significant implications. This review compares the advantages and disadvantages of using HbA_{1c} as the main diabetic diagnostic test. HbA_{1c} has greater stability and less variability than plasma glucose measurements but may not always reflect glycemic levels of glycaemia. The present cut off value identifies fewer diabetics than glucose-based criteria. HbA_{1c} being more convenient could diagnose more patients but this is not yet proven. When choosing a diagnostic test, the limitations of each test must be clearly understood to use appropriate clinical judgment and consider patient preference.

Keywords Diabetes mellitus · Diagnosis · Diabetic complications · Fasting plasma glucose · HbA_{1c} · Glycohaemoglobin · Oral glucose tolerance test

The burden of diabetes

The worldwide epidemic of diabetes is on the increase and affects 387 million people worldwide who have diabetes [1, 2]. In 2004, approximately 3.4 million people died from consequences of fasting high blood glucose [3] and most were from low and middle income countries [4]. The WHO estimates diabetes to be the seventh leading cause of death [4]. By 2025 an additional 205 million

people is estimated to have developed diabetes [5] which means that 7.7 % of the world's population are expected to be diabetic by 2030 [6]. 12 % of global health expenditure has been estimated to have been spent on diabetes in 2010 [7].

Classification of diabetes [8, 9]

Type 1 diabetes is characterized by deficiency in insulin production. The cause is under investigation and to date is not preventable. The patient experiences some or all of symptoms such as polyuria, polydipsia, polyphagia, weight loss, altered vision and exhaustion.

Type 2 diabetes results from inefficient insulin use. It affects worldwide 90 % of people with diabetes and is linked to obesity and a sedentary lifestyle. The symptoms can be similar to Type 1 but are not as prominent. Diagnosis can be several years after onset.

Gestational diabetes has been defined by the American diabetes association [9] as any degree of glucose intolerance with onset or first recognition during pregnancy. It often manifests as Type 2 diabetes and is diagnosed through prenatal screening.

Impaired glucose tolerance (IGT) and impaired fasting glycaemia (IFG) are intermediate conditions before diabetes set in and is a high risk of developing Type 2 diabetes.

Diagnosis of diabetes

Blood glucose has been used to diagnose diabetes from the twentieth century. Fasting plasma glucose (FPG) or the 2-h plasma glucose (2-h PG) value after a 75-g oral glucose tolerance test (OGTT) are used. The cut off values of

✉ Nadia Hussain
nadia.hussain@aau.ac.ae

¹ Biomedical Sciences, College of Pharmacy, Al Ain University of Science and Technology,
P.O.Box; 64141, Al Ain, UAE

glucose values differentiate between the distributions of glucose concentrations of nondiabetics.

In 2008, the American Diabetes Association organized the International Expert Committee, the European Association for the Study of Diabetes, and the International Diabetes Federation. One of the main objectives was to diagnose diabetes using HbA_{1c} in place of the current diagnostic methods. It was added as a diagnostic tool but whether HbA_{1c} (threshold 6.5 %) was superior to the other tools was left to the clinician's discretion.

Criteria [10]

- HbA_{1c} ≥ 6.5 % OR.
- FPG ≥ 126 mg/dl [7.0 mmol/L] OR.
- 2-h PG ≥ 200 mg/dl [11.1 mmol/L] during and OGTT OR.
- Random plasma glucose ≥ 200 mg/dl (11.1 mmol/L) with symptoms of hyperglycemia.

Chronic hyperglycemia has been linked to diabetic complications [11]. Long term exposure to glycaemia and laboratory measurements of it provides a better clue about the presence and severity of the disease than snapshot glucose concentration values. Long term glycaemia is measured with HbA_{1c} and has been linked to complications such as retinopathy and the range between 6.6 and 7 % is important [12]. In a study comparing fasting plasma glucose and HbA_{1c}, the link between HbA_{1c} and retinopathy was stronger than fasting glucose levels [13].

HbA_{1c} levels and complications have been correlated in clinical studies of type 1 and type 2 diabetes.

This has led to the use of HbA_{1c} goals as targets for glycaemic control. Using FPG and 2-h PG to diagnose diabetes has been debated since both measure different physiological measures of acute glucose metabolism [14].

Hemoglobin HbA_{1c}

Red blood cells contain haemoglobin [Hb] to carry oxygen around the circulatory system. Haemoglobin A_{1c} was discovered in the late 1950s [15] and Rahbar linked it to diabetes a decade later [16, 17]. Studies connected HbA_{1c} to blood glucose concentrations [18, 19]. This helped to better understand HbA_{1c} [20] and for it to be used in judging glycaemic control.

Glycation is a non-enzymatic process by which glucose binds to proteins, it occurs as a two-step Maillard formation that forms an Amadori product; HbA_{1c} [21]. Glucose rapidly diffuses into the red blood cells by the GLUT1 transporter [22]. The rate of glycation depends on how

much is the blood glucose content and HbA_{1c} levels indicate glycaemia over the last few months.

Other studies have shown the link between HbA_{1c} and a chronic state of hyperglycaemia. The HbA_{1c}-Derived Average Glucose (ADAG) study examined this with the help of continuous glucose monitoring and frequent capillary glucose measurements. HbA_{1c} was found to correlate with the average level of glucose in the blood over the preceding three month duration [23]. Since HbA_{1c} is linked to red blood cells, factors that affect them also affect HbA_{1c}. The longer a red blood cell is in circulation, the more chance it can be glycated. The average half-life of a red blood cell is approximately 30 days [24]. The last three to four months prior to HbA_{1c} testing contributes to ten per cent of the final result and the average blood glucose in the prior one month contributes to 50 % [25].

Advantages of HbA_{1c} for diagnosis

There are several advantages of using HbA_{1c} for diagnosis.

Plasma glucose levels vary throughout the day, most prominently after meals. Factors such as exercise, sleep, stress, mental activity all affect glucose. This variability is more prominent in the 2-h PG and postprandial glucose testing values. The current diagnostic criteria depends on the assumption that fasting blood glucose is reliable and relatively reproducible. However, this value is also affected by variation. In particular, exercise (late evening or early morning), a fast of less than 8 h, apprehension about testing and medications affect this value. Many patients do not consume the recommended 200 g of carbohydrate in the days before glucose is tested. The lack of appropriate preparation for testing influences the glucose levels and could reduce the reliability of using FPG for diagnosis. HbA_{1c} is not affected by sudden glucose level fluctuations or compromised fasting durations. It can be measured regardless of the time of day and in any state of fasting or absorption.

The oral glucose tolerance test is often considered the gold standard for diabetes diagnosis. However this is time-consuming, requires fasting and uses multiple samples. Considering the large number of undiagnosed diabetes cases, a simpler diagnostic test as HbA_{1c} may improve detection [25].

Fasting blood glucose levels can show more biological variability in two readings but HbA_{1c} is not as affected [26]. Even if the preparation for glucose testing is optimal, the glucose levels can be incorrect because of pre-analysis instability. Tubes used to collect blood do not always contain antiglycolytic substances and if present, there is significant glucose consumption in red blood cells in the

first 2 h of sampling because glycolysis is inhibited in its more distal steps by NaF or other preservatives. For as long as the sample is not processed or centrifuged, there is significant glucose loss. Samples reach the laboratory and are processed after some time. Glucose concentrations can show lower than actual values, a process accelerated in high temperatures [27, 28]. HbA_{1c} does not undergo such significant variability.

Clinical trial data from the Diabetes Control and Complications Trial and the United Kingdom Prospective Diabetes Study show the importance of reducing HbA_{1c} to reduce microvascular complications in type 1 and type 2 diabetes [23]. HbA_{1c} has also been linked to cardiovascular complication risks [23].

Observational studies such as DETECT-2 (evaluation of screening and early detection strategies for type 2 diabetes and impaired glucose tolerance) pooled data from over 44,000 subjects in five countries. It showed HbA_{1c} has a narrow threshold range in which complications begin to increase alarmingly. This suggests HbA_{1c} is linked to diabetic specific complications at least as much as FPG or 2-h PG [29, 30].

Standardization of the HbA_{1c} has always been a main concern but only when it is considered for diagnosis, not for monitoring diabetes as it is widely used today. Standardization helps reduce laboratory biases and is vital for HbA_{1c}—whether as a monitoring or diagnostic tool or for both. Glucose assays are considered highly reproducible across a variety of laboratories but up to 12 % of subjects have been misclassified in terms of glucose tolerance [31]. This indicates HbA_{1c} standardization need not hinder its use as a diagnostic tool. A program for worldwide implementation to equip physicians with more reliable information to monitor diabetic patients is underway [32]. Better standardization of HbA_{1c} can also be developed when HbA_{1c} is used for diagnosis.

HbA_{1c} is already used to observe metabolic control of the individual. Treatment and lifestyle is adjusted according to its value. The use of HbA_{1c} as diagnosis, ≥ 6.5 %, also allows immediate deviation detection. When measured again, after the initial diagnosis, it indicates if the metabolic control is adequate or not. This makes it easier for the physician to introduce early therapeutic interventions and lifestyle alterations. In individuals with HbA_{1c} of 5.50–5.99 % and other risk factors such as hypertension, dyslipidemia and obesity, advice can be offered because of the high risk of diabetes and a single HbA_{1c} value is more valuable than a fasting plasma glucose reading to understand the chronic values of a borderline glucose level that is borderline.

Considering cost, in terms of reagent and equipment HbA_{1c} is not cheaper than FPG. But there are other not as obvious costs. From the patient perspective, FPG requires

an overnight fast and many patients ask a friend or relative to drive to the laboratory. This can sometimes occur during work hours. HbA_{1c} is not time bound and can be measured after work hours. HbA_{1c} is also resorted to when FPG is ≥ 126 mg/dL or ≥ 7 mmol/L, requiring an additional lab. When HbA_{1c} is used initially and values are ≥ 6.5 %, the next step to monitor the diabetes after diagnosis is already complete and saves analytical and non-analytical costs. When FPG is used to screen for diabetes and a value of 5.6–6.9 mmol/L or 100–125 mg/dl [impaired glucose tolerance] is obtained, the next step is an OGTT to elucidate glucose tolerance. This needs several laboratory and adds to all costs. In these cases, where a large population is at risk or already has diabetes, HbA_{1c} instead of FPG would provide a cost effective diabetic diagnosis and risk assessment without additional, time consuming and costly testing [33].

The individual glycation variation could be an added on benefit in assessing complication risk. The hemoglobin glycation index; the difference between observed and predicted HbA_{1c} levels, can be linked to an increase in risk of developing complications [34].

The epidemiologic landscape of diabetes would alter if HbA_{1c} is picked as the diagnostic tool of choice. However a report exists indicating that using HbA_{1c} instead of FPG would not significantly change this scenario and 97.7 % of subjects would not be categorized differently. Additionally, approximately half of the subjects with a FPG ≥ 7 mmol/L (≥ 126 mg/dL) had HbA_{1c} values of 6.00–6.49 %. This meant that strict monitoring and intervention was required.

The gold standard, at present, is the combination of FPG, 2 h-PG and HbA_{1c} but is not feasible or recommended for a variety of reasons especially on a large scale. HbA_{1c} appears more feasible.

Issues with HbA_{1c} to diagnose diabetes

There has been much debate about using HbA_{1c} to diagnose diabetes. One concern is that HbA_{1c} is a measure of glycation and not a direct measurement of glycaemia. There are many physiological and pathological states influencing HbA_{1c}. HbA_{1c} is altered when three basic mechanisms are affected; amount of glucose entering red blood cells, red blood cell age in circulation and the glycation rate.

There is heterogeneity in the glucose concentration gradient across the red blood cell membrane and the average lifespan of red blood cells, even in healthy individuals. In pathological states affecting red blood cell turnover, such as an increase or decrease in erythropoiesis with or without haemolysis, HbA_{1c} levels are affected. Care has to be taken when analyzing HbA_{1c} results from

patients who have liver or renal failure or haemoglobinopathies.

HbA_{1c} has also faced concerns that it will identify a population set of diabetics different from those that have been identified by measurements of plasma glucose. The objective of any diagnostic criteria is to identify people at risk of complications and would benefit from interventions, not to detect people with particular plasma glucose values. However, multiple criteria for diagnosing can create confusion.

HbA_{1c} may vary with age and ethnicity [35]. People of African ethnicity may have higher HbA_{1c} levels than Caucasians [35, 36]. Evidence indicates HbA_{1c} levels correlate differently with plasma glucose amongst South Asians or Inuits compared to Caucasians [23]. The reasons are unknown but differences in genetic determinants of haemoglobin glycation, the glucose balance between extracellular and intracellular environment and red blood cell turn over are considered [37]. This might necessitate ethnicity specific thresholds to use HbA_{1c} for diagnosis.

Abnormal haemoglobin levels could affect HbA_{1c} values and depending on the method used, not all assays can differentiate between pathological and glycated haemoglobin. Different races exhibit different frequencies of haemoglobin disorders; 10 % of African-Americans have a haemoglobin C trait that could interfere with HbA_{1c} assay [38]. The National Glycohemoglobin Standardization Program website (NGSP, <http://www.ngsp.org/interf.asp>) provides current information on how the most common haemoglobin variants such as HbS, HbC and HbE interfere with twenty methods that measure HbA_{1c}, although some methods remain unaffected. However, if the patient is a homozygous or double heterozygous for a haemoglobin variant then no method can measure HbA_{1c} because it is not present.

Standardization of measuring HbA_{1c} has been improved by adopting the NGSP protocols but not all countries have achieved this. Plasma glucose concentration is also difficult to assay with consistent accuracy. It is estimated that up to 12 % of patients can be misclassified in the diagnosis of diabetes due to laboratory instrument error in measuring glucose [31, 39].

Depending on the study, the probability of a person meeting the plasma glucose criteria for diabetes and having an HbA_{1c} ≥ 6.5 % can range between 17–78 % [40]. The discordance is wide and varies between different populations [23]. The different methodologies used can partly explain the difference but even so if HbA_{1c} becomes used globally, the epidemiology of type 2 diabetes mellitus would be altered.

The diagnosis of diabetes based on HbA_{1c} misses a large proportion of asymptomatic early cases of diabetes that are only detected by the OGTT. FPG has shown to be

more sensitive than HbA_{1c} [41]. People with impaired glucose tolerance cannot be detected by HbA_{1c} and it is this group that can benefit most by diabetes prevention programs [42, 43]. This is of particular importance when considering evidence that this group has an ~ 40 % chance of increased mortality [44]. Early diagnosis of diabetes is of the utmost importance.

Studies show using HbA_{1c} and FPG and/or OGTT identify different diabetic groups [45]. HbA_{1c} ≥ 6.5 % identifies 30–40 % of previously undiagnosed diabetics but FPG estimates 50 % and the OGTT detects 90 % of diabetics [46, 47].

A large proportion of newly diagnosed based on current glucose criteria have HbA_{1c} ≤ 6.5 %. In the Finnish Diabetes Prevention study, the sensitivity of HbA_{1c} ≥ 6.5 % to diagnose diabetes was 39 % so 61 % of newly diagnosed diabetics had an HbA_{1c} ≤ 6.5 % [48]. This means that 61 % of patients with diabetes could be diagnosed later and risk complications.

The decision on a single, optimal threshold for HbA_{1c} values has been more difficult than expected. The expert committee recommended a cut-point of 6.5 % and was based on evidence that in diverse populations, retinopathy seemed to increase above this particular level [49]. However, this recommendation was based on an unpublished analysis of data derived from the DETECT-2 study and cited as a personal communication by one of the committee members, Stephen Colagiuri [9, 50]. Another article that discussed the DETECT-2 study clearly pointed out that “currently available statistical and mathematical models were not able to identify a clear cut-point. It also states that the cut-points for diabetes are a combination of the available evidence and expert consensus [51].

Statements such as these summarize the dilemma posed when a cut-point for diabetes is selected which is based on retinopathy. The expert committee [49] pointed towards older studies that analyzed the relationship of retinopathy to FPG, 2-h PG and HbA_{1c} [52, 53]. These studies observed that retinopathy appeared to increase when HbA_{1c} levels of 6 % in the National Health and Nutrition Examination Survey (NHANES) III, 6.2 % in the Pima Indian Study and 6.3 % in a study done in the Egyptian population [54]. The 1066 people studies as part of the 2005–2006 NHANES cohort showed that increased retinopathy at A1C ≥ 5.5 % [55]. A Japanese study has also suggested that the optimal cut-point should be 5.3–5.5 % [56]. A South Asian study pointed out the concept of a threshold effect. The authors of this study report a continuous linear relationship between HbA_{1c} and the most common microvascular complications. It was found, in the population studies, that mild to moderate retinopathy was rare below an HbA_{1c} of 6.6–7 %. This study concluded that an HbA_{1c} of 6.6 % may represent an

optimal cut-point for diagnosing diabetes based on the risk of mild to moderate retinopathy but not for other microvascular complications [12]. A review of three population-based cross-sectional studies of the relationship between retinopathy and FPG concluded that there is “inconsistent evidence” for a uniform glycemia threshold for prevalent and incident retinopathy. The analyses also suggested the existence of a continuous relationship between glucose levels and retinopathy, including moderate retinopathy [57]. The Multi-Ethnic Study of Atherosclerosis (MESA), also examined the relationship between HbA_{1c} and retinopathy and found a continuous, non-inflected relationship between these two parameters with no threshold effect [57].

The discrepancies amongst studies about the threshold effect could be partly due to older studies relying on direct ophthalmoscopy or single field retinal photographs which are not as reliable as new methods for detecting early stage retinopathy [58]. There are also ethnic groups that show a higher prevalence of retinopathy at relatively lower glycemia levels. An Australian study showed that individuals who had not been diagnosed with diabetes and had retinopathy, had a higher risk of being diagnosed with diabetes 5 years later [29]. This suggests that retinopathy can start at glucose concentrations below the threshold for the diagnosis of diabetes. The association of retinopathy with “pre-diabetes” was also observed in the Diabetes Prevention Program [59] and in the 2005–2006 NHANES study; both studies found that 8 % of individuals with FPG levels below the diagnostic threshold for diabetes had retinopathy [60].

HbA_{1c} also has issues when it is correlated with glycaemia in ‘non-diabetic’ populations. The Dutch New Hoorn study found that HbA_{1c} correlated well with FPG and 2-h PG in people with diabetes diagnosed by criteria before 2010. However, the correlation of HbA_{1c} with FPG and 2-h PG in the general population was significantly lower. The authors concluded that HbA_{1c} is affected by aging, genetic traits, differences in red blood cell lifespan especially for non-diabetic individuals [61].

Glyco-oxidation products and glycated products are present on α and β globins but are more abundant in diabetics. The methods measuring glycated haemoglobin cannot distinguish between glycated and glyco-oxidated species on HbA_{1c} [62]. In type 2 diabetics with chronic complications there is a higher abundance of glyco-oxidation products which might affect the diagnosis of diabetes [63].

2-h PG and HbA_{1c} are better predictors than FPG for future cardiovascular complications but derived by multivariate analysis, only 2-h PG remains statistically important [23].

In terms of expense, HbA_{1c} is an expensive test. Many patients also require other tests such as lipid or hepatic profile during the fasting state, at the same time, and adding

glucose is not a major issue. Most laboratories can test before average working hours.

HbA_{1c} is unaffordable or even unavailable in many low and middle income countries [64, 65]. Setting HbA_{1c} as the gold standard for diagnosing diabetes creates a divide between societies that have or have no access to HbA_{1c} testing.

Conclusion

Diabetes places a heavy global burden of disease and the definition of it is of the utmost importance. HbA_{1c} has many attractive attributes and can be used as an appropriate diagnostic test when an internationally standardized assay is used and clinical conditions that affect it are accounted for. When a diagnostic test is used for diabetes, the limitations of the choices currently available must be clearly understood. The use of clinical judgment and patient preference are needed to select the appropriate test.

Until the challenges against using HbA_{1c} have not been better met, using HbA_{1c} as the sole diagnostic test could potentially lead to misclassification and errors. It must be used thoughtfully and preferably in combination with traditional glucose criteria when screening for and diagnosing diabetes.

Compliance with ethical standards

Ethics policy This article does not contain any studies with human or animal subjects performed by any of the authors.

Conflict of interest The author declares that they have no conflict of interest.

References

1. Danaei G, Finucane MM, Lu Y, Singh GM, Cowan MJ, Paciorek CJ, et al. National, regional, and global trends in fasting plasma glucose and diabetes prevalence since 1980: systematic analysis of health examination surveys and epidemiological studies with 370 country-years and 2.7 million participants. *Lancet*. 2011;378(9785):31–40.
2. Guariguata L, Whiting D, Hambleton I, Beagley J, Linnenkamp U, Shaw J. Global estimates of diabetes prevalence for 2013 and projections for 2035. *Diabetes Res Clin Pract*. 2014;103(2):137–49.
3. World Health Organization. Global health risks: mortality and burden of disease attributable to selected major risks. Geneva: World Health Organization; 2009. Retrieved from http://www.who.int/healthinfo/global_burden_disease/publications/en/.
4. Global status report on noncommunicable diseases. Description of the global burden of NCDs, their risk factors and determinants. Geneva: World Health Organization; 2010. http://www.who.int/nmh/publications/ncd_report2010/en/.
5. International Diabetes Federation. IDF Diabetes Atlas. 6th ed. Brussels, Belgium: International Diabetes Federation; 2010. <http://www.idf.org/diabetesatlas>.

6. Shaw JE, Sicree RA, Zimmet PZ. Global estimates of the prevalence of diabetes for 2010 and 2030. *Diabetes Res Clin Pract.* 2010;87(1):4–14.
7. Zhang P, Zhang X, Brown J, Vistisen D, Sicree R, Shaw J, et al. Global healthcare expenditure on diabetes for 2010 and 2030. *Diabetes Res Clin Pract.* 2010;87(3):293–301.
8. World Health Organization. Definition, diagnosis and classification of diabetes mellitus and its complications: report of a consultation. Part 1, Diagnosis and classification of diabetes mellitus. Geneva: World Health Organization; 1999. <http://apps.who.int/iris/handle/10665/66040>.
9. Association AD. Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care.* 2014;37(Supplement 1):S81–90.
10. Association AD. Standards of medical care in diabetes—2014. *Diabetes Care.* 2014;37(Supplement 1):S14–80.
11. Fowler MJ. Microvascular and macrovascular complications of diabetes. *Clin Diabetes.* 2008;26(2):77–82.
12. Sabanayagam C, Liew G, Tai ES, Shankar A, Lim SC, Subramaniam T, et al. Relationship between glycated haemoglobin and microvascular complications: is there a natural cut-off point for the diagnosis of diabetes? *Diabetologia.* 2009;52(7):1279–89.
13. Tapp RJ, Tikellis G, Wong TY, Harper CA, Zimmet PZ, Shaw JE. Longitudinal association of glucose metabolism with retinopathy: results from the Australian diabetes obesity and lifestyle (AusDiab) study. *Diabetes Care.* 2008;31(7):1349–54.
14. Abdul-Ghani MA, Jenkinson CP, Richardson DK, Tripathy D, DeFronzo RA. Insulin secretion and action in subjects with impaired fasting glucose and impaired glucose tolerance results from the veterans administration genetic epidemiology study. *Diabetes.* 2006;55(5):1430–5.
15. Allen DW, Schroeder W, Balog J. Observations on the chromatographic heterogeneity of normal adult and fetal human hemoglobin: a study of the effects of crystallization and chromatography on the heterogeneity and isoleucine content. *J Am Chem Soc.* 1958;80(7):1628–34.
16. Rahbar S. An abnormal hemoglobin in red cells of diabetics. *Clin Chim Acta.* 1968;22(2):296–8.
17. Rahbar S, Blumenfeld O, Ranney HM. Studies of an unusual hemoglobin in patients with diabetes mellitus. *Biochem Biophys Res Commun.* 1969;36(5):838–43.
18. Koenig RJ, Peterson CM, Jones RL, Saudek C, Lehrman M, Cerami A. Correlation of glucose regulation and hemoglobin A_{1c} in diabetes mellitus. *N Engl J Med.* 1976;295(8):417–20.
19. Koenig RJ, Peterson CM, Kilo C, Cerami A, Williamson JR. Hemoglobin A_{1c} as an indicator of the degree of glucose intolerance in diabetes. *Diabetes.* 1976;25(3):230–2.
20. Koenig RJ, Cerami A. Hemoglobin A_{1c} and diabetes mellitus. *Annu Rev Med.* 1980;31:29–34.
21. Gallagher EJ, Le Roith D, Bloomgarden Z. Review of hemoglobin A_{1c} in the management of diabetes. *J Diabetes.* 2009;1(1):9–17.
22. Carruthers A. Facilitated diffusion of glucose. *Physiol Rev.* 1990;70(4):1135–76.
23. Linters-Westra E, Slingerland RJ. Hemoglobin A_{1c} determination in the A1C-Derived Average Glucose (ADAG) study. *Clin Chem Lab Med.* 2008;46(11):1617–23.
24. Jeffcoat S. Diabetes control and complications: the role of glycated haemoglobin, 25 years on. *Diabet Med.* 2004;21(7):657–65.
25. Borch-Johnsen K, Colagiuri S. Diagnosing diabetes—time for a change? *Diabetologia.* 2009;52(11):2247–50.
26. Rohlfing C, Wiedmeyer H-M, Little R, Grotz VL, Tennill A, England J, et al. Biological variation of glycohemoglobin. *Clin Chem.* 2002;48(7):1116–8.
27. Mikesch LM, Bruns DE. Stabilization of glucose in blood specimens: mechanism of delay in fluoride inhibition of glycolysis. *Clin Chem.* 2008;54(5):930–2.
28. Bruns DE, Knowler WC. Stabilization of glucose in blood samples: why it matters. *Clin Chem.* 2009;55(5):850–2.
29. Tapp RJ, Tikellis G, Wong TY, Harper CA, Zimmet PZ, Shaw JE. Longitudinal association of glucose metabolism with retinopathy results from the Australian diabetes obesity and lifestyle (AusDiab) Study. *Diabetes Care.* 2008;31(7):1349–54.
30. Colagiuri S, Lee CM, Wong TY, Balkau B, Shaw JE, Borch-Johnsen K. Glycemic thresholds for diabetes-specific retinopathy implications for diagnostic criteria for diabetes. *Diabetes Care.* 2011;34(1):145–50.
31. Miller WG, Myers GL, Ashwood ER, Killeen AA, Wang E, Ehlers GW, et al. State of the art in trueness and interlaboratory harmonization for 10 analytes in general clinical chemistry. *Arch Pathol Lab Med.* 2008;132(5):838–46.
32. Consensus Committee. Consensus statement on the worldwide standardization of the hemoglobin A_{1c} measurement: the American Diabetes Association, European Association for the Study of Diabetes, International Federation of clinical chemistry and laboratory medicine, and the International Diabetes Federation. *Diabetes Care.* 2007;30(9):2399.
33. Selvin E, Steffes MW, Zhu H, Matsushita K, Wagenknecht L, Pankow J, et al. Glycated hemoglobin, diabetes, and cardiovascular risk in nondiabetic adults. *N Engl J Med.* 2010;362(9):800–11.
34. McCarter RJ, Hempe JM, Gomez R, Chalew SA. Biological variation in HbA_{1c} predicts risk of retinopathy and nephropathy in type 1 diabetes. *Diabetes Care.* 2004;27(6):1259–64.
35. Davidson MB, Schriger DL. Effect of age and race/ethnicity on HbA_{1c} levels in people without known diabetes mellitus: implications for the diagnosis of diabetes. *Diabetes Res Clin Pract.* 2010;87(3):415–21.
36. Ziemer DC, Kolm P, Weintraub WS, Vaccarino V, Rhee MK, Twombly JG, et al. Glucose-independent, black-white differences in hemoglobin A_{1c} levels: a cross-sectional analysis of 2 studies. *Ann Intern Med.* 2010;152(12):770–7.
37. Herman WH, Cohen RM. Racial and ethnic differences in the relationship between HbA_{1c} and blood glucose: implications for the diagnosis of diabetes. *J Clin Endocrinol Metab.* 2012;97(4):1067–72.
38. Roberts WL, Safar-Pour S, De BK, Rohlfing CL, Weykamp CW, Little RR. Effects of hemoglobin C and S traits on glycohemoglobin measurements by eleven methods. *Clin Chem.* 2005;51(4):776–8.
39. Gambino R. Glucose: a simple molecule that is not simple to quantify. *Clin Chem.* 2007;53(12):2040–1.
40. Christensen DL, Witte DR, Kaduka L, Jørgensen ME, Borch-Johnsen K, Mohan V, et al. Moving to an A1C-based diagnosis of diabetes has a different impact on prevalence in different ethnic groups. *Diabetes Care.* 2010;33(3):580–2.
41. Zhou X, Pang Z, Gao W, Wang S, Zhang L, Ning F, et al. Performance of an A1C and fasting capillary blood glucose test for screening newly diagnosed diabetes and pre-diabetes defined by an oral glucose tolerance test in Qingdao, China. *Diabetes Care.* 2010;33(3):545–50.
42. Tuomilehto J, Lindström J, Eriksson JG, Valle TT, Hämäläinen H, Ilanne-Parikka P, et al. Prevention of type 2 diabetes mellitus by changes in lifestyle among subjects with impaired glucose tolerance. *N Engl J Med.* 2001;344(18):1343–50.
43. Eriksson K-F, Lindgärde F. No excess 12-year mortality in men with impaired glucose tolerance who participated in the Malmö preventive trial with diet and exercise. *Diabetologia.* 1998;41(9):1010–6.
44. Uusitupa M, Peltonen M, Lindström J, Aunola S, Ilanne-Parikka P, Keinänen-Kiukkaanniemi S, et al. Ten-year mortality and cardiovascular morbidity in the Finnish diabetes prevention study—secondary analysis of the randomized trial. *PLoS One.* 2009;4(5):e5656.
45. Cowie CC, Rust KF, Byrd-Holt DD, Gregg EW, Ford ES, Geiss LS, et al. Prevalence of diabetes and high risk for diabetes using

- A1C criteria in the US population in 1988–2006. *Diabetes Care*. 2010;33(3):562–8.
46. Carson AP, Reynolds K, Fonseca VA, Muntner P. Comparison of A1C and fasting glucose criteria to diagnose diabetes among US adults. *Diabetes Care*. 2010;33(1):95–7.
 47. Lorenzo C, Wagenknecht LE, Hanley AJ, Rewers MJ, Karter AJ, Haffner SM. A1C Between 5.7 and 6.4% as a marker for identifying pre-diabetes, insulin sensitivity and secretion, and cardiovascular risk factors the insulin resistance atherosclerosis study (IRAS). *Diabetes Care*. 2010;33(9):2104–9.
 48. Pajunen P, Peltonen M, Eriksson J, Ilanne-Parikka P, Aunola S, Keinänen-Kiukaanniemi S, et al. HbA1c in diagnosing and predicting type 2 diabetes in impaired glucose tolerance: the finnish diabetes prevention study. *Diabet Med*. 2011;28(1):36–42.
 49. Committee IE. International expert committee report on the role of the A1C assay in the diagnosis of diabetes. *Diabetes Care*. 2009;32(7):1327–34.
 50. Colagiuri S, Borch-Johnsen K. DETECT-2: early detection of type 2 diabetes and IGT. *Diabetes Voice*. 2003;48(4):11–3.
 51. Borch-Johnsen K, Colagiuri S. Diagnosing diabetes—time for a change? *Diabetologia*. 2009;52(11):2247–50.
 52. McCance DR, Hanson RL, Charles MA, Jacobsson LT, Pettitt DJ, Bennett PH, et al. Comparison of tests for glycated haemoglobin and fasting and two hour plasma glucose concentrations as diagnostic methods for diabetes. *BMJ*. 1994;308(6940):1323–8.
 53. Engelgau MM, Thompson TJ, Herman WH, Boyle JP, Aubert RE, Kenny SJ, et al. Comparison of fasting and 2-hour glucose and HbA1c levels for diagnosing diabetes. Diagnostic criteria and performance revisited. *Diabetes Care*. 1997;20(5):785–91.
 54. Davidson MB, Schriger DL, Peters AL, Lorber B. Relationship between fasting plasma glucose and glycosylated hemoglobin—potential for false-positive diagnoses of type 2 diabetes using new diagnostic criteria. *JAMA*. 1999;281(13):1203–10.
 55. Cheng YJ, Gregg EW, Geiss LS, Imperatore G, Williams DE, Zhang X, et al. Association of A1C and fasting plasma glucose levels with diabetic retinopathy prevalence in the U.S. population: implications for diabetes diagnostic thresholds. *Diabetes Care*. 2009;32(11):2027–32.
 56. Miyazaki M, Kubo M, Kiyohara Y, Okubo K, Nakamura H, Fujisawa K, et al. Comparison of diagnostic methods for diabetes mellitus based on prevalence of retinopathy in a Japanese population: the Hisayama study. *Diabetologia*. 2004;47(8):1411–5.
 57. Wong TY, Liew G, Tapp RJ, Schmidt MI, Wang JJ, Mitchell P, et al. Relation between fasting glucose and retinopathy for diagnosis of diabetes: three population-based cross-sectional studies. *Lancet*. 2008;371(9614):736–43.
 58. van Leiden HA, Moll AC, Dekker JM, Abramoff MD, Polak BC. Photography or ophthalmoscopy for detection of diabetic retinopathy? *Diabetes Care*. 2003;26(4):1318–9.
 59. Group DPPR. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. *N Engl J Med*. 2002;346(6):393.
 60. Cohen RM, Smith EP. Frequency of HbA1c discordance in estimating blood glucose control. *Curr Opin Clin Nutr Metab Care*. 2008;11(4):512–7.
 61. van't Riet E, Alssema M, Rijkkelijkhuizen JM, Kostense PJ, Nijpels G, Dekker JM. Relationship between A1C and glucose levels in the general Dutch population: the new Hoorn study. *Diabetes Care*. 2010;33(1):61–6.
 62. Lapolla A, Tubaro M, Fedele D, Reitano R, Arico N, Ragazzi E, et al. A matrix-assisted laser desorption/ionization mass spectrometry study of the non-enzymatic glycation products of human globins in diabetes. *Rapid Commun Mass Spectrom*. 2005;19(2):162–8.
 63. Lapolla A, Mosca A, Fedele D. The general use of glycated haemoglobin for the diagnosis of diabetes and other categories of glucose intolerance: still a long way to go. *Nutr Metab Cardiovasc Dis*. 2011;21(7):467–75.
 64. World Health Organization. Use of glycated haemoglobin (HbA1c) in diagnosis of diabetes mellitus: abbreviated report of a WHO consultation. Geneva: World Health Organization; 2011. <http://apps.who.int/iris/handle/10665/70523>.
 65. Mathers CD, Loncar D. Projections of global mortality and burden of disease from 2002 to 2030. *PLoS Med*. 2006;3(11):e442.