Macrovascular Risk and Diagnostic Criteria for Type 2 Diabetes

Implications for the use of FPG and HbA1c for cost-effective screening

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OBJECTIVE — The use of fasting plasma glucose (FPG) level ≥7.0 mmol/l leads to under-diagnosis of type 2 diabetes compared with the oral glucose tolerance test (OGTT). The OGTT is of limited use for population screening. Most of the increase in cardiovascular risk in relation to increasing blood glucose occurs before the threshold at which the diagnosis of type 2 diabetes is made. The aim of this study was to evaluate the use of HbA1c, FPG and HbA1c as predictors of type 2 diabetes and cardiovascular risk and, accordingly, to develop a rational approach to screening for abnormalities of glucose tolerance.

RESEARCH DESIGN AND METHODS — OGTT and measurement of HbA1c and FPG levels were performed in 505 subjects screened for type 2 diabetes. Anthropomorphic measurements were obtained. A cardiovascular risk factor questionnaire was completed.

RESULTS — The subjects were aged 19–88 years (mean 53.8). The incidence of type 2 diabetes was 10.4% based on the OGTT and 4% based on an FPG level ≥7.0 mmol/l. Using high-performance liquid chromatography (HPLC), HbA1c of <4.7 and ≥6.2% predicted with certainty the absence or presence of type 2 diabetes as defined by the OGTT. The corresponding cutoffs were ≤5.0 and ≥6.8% for HbA1c (DCA2000 HPLC device; Bayer Diagnostics, Mulgrave, Australia) and <4.7 and ≥6.4 mmol/l for FPG. However, 73–85% of subjects in each case had intermediate values, which were therefore nondiagnostic. Cardiovascular risk increased at least 2.2 times at an HbA1c level ≥6.2% (by HPLC), 1.8–2.2 times at an HbA1c level of 5.6–6.1% (by HPLC), 2 times at an FPG level ≥6.4 mmol/l, and 1.7–1.9 times at an FPG level of 5.6–6.3 mmol/l.

CONCLUSIONS — Measurement of FPG and HbA1c levels will diagnose or exclude type 2 diabetes with certainty in a minority (15%) of people. There is a continuous relationship between FPG and HbA1c, and cardiovascular risk. Accordingly, we propose that there is a rational basis for using either FPG and HbA1c for purposes of screening and assigning risk. Individuals with an HbA1c level of 5.6–6.1% and an FPG level of 5.6–6.3 mmol/l are at greatest risk for cardiovascular disease and should be targeted for further evaluation. An algorithm outlining a cost-effective approach is presented.


The incidence of type 2 diabetes varies from 3.6 to 14.8%, depending on the population being screened (1,2) and the diagnostic criteria used. Up to 50% of diabetic subjects remain undetected. The oral glucose tolerance test (OGTT) is the gold standard for diagnosing type 2 diabetes. It has limited use for mass screening, due to the need for fasting, the time-consuming nature of the test, and poor reproducibility of the results (3,4). The American Diabetes Association (ADA) based diagnosis of diabetes on a fasting plasma glucose (FPG) level of 7.0 mmol/l because this level correlates with a 2-h (post–75 g glucose) level of ≥11.1 mmol/l (5). It was hoped the better reproducibility of FPG and its relative convenience would increase the number of diabetic subjects diagnosed. The ADA also created a new category termed impaired fasting glycemia (IFG) to describe patients with FPG levels of 6.1–6.9 mmol/l (5) to categorize individuals at increased risk for type 2 diabetes and those who may be at increased cardiovascular risk. The World Health Organization (WHO) and subsequently the Australian Diabetes Society similarly adopted an FPG level of 7 mmol/l as the threshold for diagnosing type 2 diabetes (6,7); however, these organizations continue to recommend use of the OGTT, because patients with type 2 diabetes based on an OGTT often have a nondiabetic FPG level (1,8,9).

To improve the detection rate of type 2 diabetes, alternative approaches to screening have been proposed, such as a lower threshold for FPG (8) or the use of HbA1c (4,10–12). Measurement of HbA1c is used to determine average glycemic control over an 8- to 12-week period, and HbA1c level has been linked to development of microvascular complications such as neuropathy, nephropathy, and retinopathy (7). Compared with the OGTT, HbA1c measurement is quicker, is more convenient, and avoids the need for fasting (3). Problems with the use of HbA1c for screening have included variability and poor standardization of assays, biological variability of HbA1c levels, overlap between subjects with and without diabetes as compared with fasting or 2-h glucose levels (5,14–17), and poor sensitivity (12). When measured using high-performance liquid chromatography (HPLC), however, the test has high precision (interassay coefficient of variation [CV] 1–2%). Furthermore, rapid, on-
Macrovascular risk and diagnostic criteria for type 2 diabetes

the-spot results comparable to those obtained with HPLC can be obtained using automated and portable devices (17).

The current OGTT and FPG thresholds for diagnosis of diabetes are based on their association with microvascular disease, the incidence of which increases sharply above currently defined glycemic thresholds. Macrovascular disease, however, seems to increase gradually in prediabetic states such as impaired glucose tolerance (IGT) and IFG (4,18). Given that a major part of the morbidity and mortality from type 2 diabetes arises from macrovascular disease such as ischemic heart disease and not just microvascular disease, any screening test for diabetes would be more meaningful if it could also predict cardiovascular disease. We hypothesized that levels of HbA1c may increase progressively with increasing plasma glucose levels, even below conventionally defined diabetic thresholds, and are associated with the risk of macrovascular disease.

The aims of this study were 1) to compare the utility of HbA1c and FPG at different thresholds as screening tests for diagnosing type 2 diabetes, as defined by OGTT criteria, 2) to determine the relationship between HbA1c and FPG and cardiovascular risk; and 3) to compare HbA1c measured by HPLC with the result obtained using a portable device (DCA2000; Bayer Diagnostics, Mulgrave, Australia) to assess the potential utility of the latter in screening for type 2 diabetes.

RESEARCH DESIGN AND METHODS

Subjects
Subjects were recruited for this study by community advertisement for people with obesity, family history of diabetes, history of gestational diabetes, or symptoms such as polyuria and polydipsia. All individuals older than 18 years of age without a previous diagnosis of type 2 diabetes were tested if they responded to the advertisement with a request for screening. Pregnant women were excluded from the study. Patients referred by general practitioners and other hospital specialists for an OGTT were offered the opportunity to participate in the study. To determine reproducibility of the tests used, 41 subjects were tested on two occasions (1 week apart). The Ethics Committee of the Royal Adelaide Hospital approved the protocol. Informed consent was obtained from all volunteers.

Study design
Subjects fasted from food and fluid from 11:00 P.M. the previous night and attended the Endocrine Test Unit at the Royal Adelaide Hospital between 8:00 A.M. and 9:00 A.M. Subjects were asked to complete a questionnaire to document the presence of ischemic heart disease, hypertension, and hyperlipidemia and whether there was a family history of type 2 diabetes. Height, weight, and waist circumference were measured. A forearm vein was then cannulated with a 19-g butterfly and 5 ml of venous blood was collected for measurement of glucose and HbA1c. Thereafter, 75 g of glucose was administered orally and 5 ml of blood was collected at 120 min for measurement of plasma glucose. All patients and their general practitioners were sent a letter informing them of the results, and patients were advised by telephone and by letter to seek follow-up whenever either diabetes or IGT was detected.

Assays
Plasma glucose was measured by the hexokinase method, which has an interassay CV of 1.9% at a glucose level of 4.8 mmol/l. HbA1c was measured by HPLC using a spherical cation exchange gel, which has an interassay CV of 2% at an HbA1c level of 6%. HbA1c results from our laboratory (Institute of Medical and Veterinary Science) were referenced to the National Glycohemoglobin Standardisation Program. HbA1c was also measured using the DCA2000 (Bayer Diagnostics), a portable device that uses an immunoassay technique with a monoclonal antibody directed against a sequence of the HbA1c molecule (19). A result is available within 6 min of testing, with a CV of 2.2%.

Statistical analysis
Results are presented as means ± SD. The receiver operating characteristic (ROC) curve was used to describe the ability of HbA1c (HPLC or DCA2000) and FPG to determine the presence or absence of type 2 diabetes as defined by the OGTT. The ROC describes the diagnostic properties of a test by plotting sensitivity as a function of 1-specificity (20). Sensitivity is the fraction of individuals at or above the HbA1c cutoff point who have diabetes, whereas specificity is the fraction of individuals with an HbA1c level below the cutoff point who do not have diabetes. The area under the ROC curve represents the probability that a subject chosen at random from the group with the outcome of interest (type 2 diabetes) had a higher value than a subject without. Wald x^2 analysis was used to compare the respective areas under the curve for the different diabetic screening tests. The relationship between HbA1c as measured by HPLC and using the DCA2000 was determined using linear regression.

Log-binomial regression was used to determine the risk ratios (and 95% CIs) for the presence of ischemic heart disease for each SD increase in HbA1c, as measured by either HPLC or using the DCA2000 method and the FPG. A risk of 1 was arbitrarily assigned to a cutoff 2 SD below the mean for each of the aforementioned measurements, and risk ratios were then determined for higher levels. The risk ratio was also calculated for each parameter after adjusting for the presence of the other stated risk factors. The reproducibility of measurements of FPG, 2-h glucose, and HbA1c by each method was calculated for the 41 subjects, who were tested twice, and the intrasubject CV was determined (15).

RESULTS — A total of 505 subjects aged 19–88 years (mean age 53.8 years) were studied. There were 294 (58.2%) women and 211 (41.8%) men. Using the Australian Diabetes Society and WHO criteria, the incidence of type 2 diabetes was 10.7% (54 subjects) and the incidence of IGT was 24.4% (123 subjects). Using the ADA criteria, the incidence of type 2 diabetes was 4.0% (20 subjects) and the incidence of IFG was 7.1% (36 subjects).

Subjects were divided according to the presence or absence of diabetes according to OGTT criteria. Nondiabetic subjects had a mean HbA1c (HPLC) of 5.2% (range 3.5–6.6), a mean HbA1c (DCA2000) of 5.5% (4–6.9), and a mean FPG of 5.1 mmol/l (3–6.7). Diabetic subjects had a mean HbA1c (HPLC) of 6.5% (4.7–12.2), a mean HbA1c (DCA2000) of 7.0% (5–13.1), and a mean FPG of 7.4 mmol/l (4.7–15.7). There was a good correlation between the HbA1c values obtained by HPLC and DCA2000 (R^2 = 0.876). The regression data for HbA1c by DCA2000 versus HPLC reveals a small in-
HbA1c as measured by HPLC and under the curve (predictive values) of criteria showed higher values for DCA2000. Based on our ROC curves, our cutoff criteria showed higher values for DCA2000 than HPLC.

Based on an ROC analysis, the areas under the curve (predictive values) of HbA1c as measured by HPLC and DCA2000 for detecting type 2 diabetes, compared with OGTT, were 0.893 and 0.911, respectively (χ² = 0.53, df = 2, P = 0.77) (Fig. 1).

When measured by HPLC, HbA1c ≥5.7% predicted type 2 diabetes with a sensitivity of 80.0% and a specificity of 86.3%. HbA1c levels <4.7 and ≥6.2% have almost 100% accuracy for predicting the absence and presence of type 2 diabetes, respectively (Table 1).

When measured by DCA2000, HbA1c ≥6.6% (sensitivity 72.2%, specificity 94.7%) was the best predictor of diabetes, and HbA1c levels ≤5.0 and ≥6.8% predict the absence or presence of diabetes, respectively, with almost 100% certainty (Table 1). Using FPG, the area under the ROC curve was 0.9065. FPG ≥6.0 mmol/l (sensitivity 74.1%, specificity 94.5%) was the best predictor of type 2 diabetes as defined by OGTT. FPG levels <4.7 and ≥6.4 mmol/l had almost 100% accuracy for detecting the absence or presence of type 2 diabetes.

**Cardiovascular risk**

The mean ± SD for HbA1c by HPLC, HbA1c by DCA2000, and FPG were 5.3 ± 0.74%, 5.6 ± 0.84%, and 5.3 ± 1.2 mmol/l, respectively. The risk ratio (95% CI) for each SD change in HbA1c by HPLC, HbA1c by DCA2000, and FPG were 1.3 (1.1–1.5; P = 0.0002), 1.24 (1.1–1.4; P = 0.0038), and 1.26 (1.1–1.4; P = 0.0001), respectively. The relative cardiovascular risks associated with different cutoffs of HbA1c (HPLC and DCA2000) and FPG are shown in Table 1.

Neither HbA1c (HPLC or DCA2000) nor FPG remained independent risk factors for cardiovascular disease after adjustment for age, waist circumference, hypertension, and high cholesterol. After adjustment for these risk factors, the relative risk of ischemic heart disease for HbA1c by HPLC, HbA1c by DCA2000, or FPG was 1.1 (0.9–1.4; P = 0.22), 1.1 (0.8–1.4; P = 0.64), and 1.2 (0.99–1.4; P = 0.059). Only age and high cholesterol were independent risk factors for cardiovascular disease. For example, when analyzed with HbA1c (HPLC) the relative risk of ischemic heart disease for age was 2.0 (1.4–2.9; P = 0.0002) and for high cholesterol was 2.2 (1.2–3.9; P = 0.01). The ischemic heart disease risk for waist circumference was 1.2 (0.9–1.6; P = 0.18) and for hypertension was 1.7 (0.8–3.3; P = 0.14).

**Reproducibility**

There was a within-subject CV of 2.2% for HbA1c by HPLC, 2.7% for HbA1c by DCA2000, 4.9% for FPG, and 16.0% for 120-min plasma glucose after a 75-g oral glucose load.

**CONCLUSIONS**—These results show that FPG and HbA1c (by either method) will diagnose or exclude diabetes with certainty in only a minority (15%) of subjects when the OGTT, with currently defined cutoffs, is used as the gold standard. Although FPG and HbA1c do not seem to be independent measures of cardiovascular risk, there is a continuous relationship between both of these measures and cardiovascular risk. Moreover, data from the Diabetes Control and Complications Trial (DCCT) (21) and U.K. Prospective Diabetes Study (UKPDS) (22) demonstrate that there remains a significant risk of microvascular disease with HbA1c levels well below 8%, and even at an HbA1c of 6%, there is a 75%

Table 1—The sensitivity, specificity, and cardiovascular risk ratio at each cutoff of HbA1c (by HPLC), HbA1c (by DCA2000), and FPG

<table>
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<tr>
<th>HbA1c (%)</th>
<th>Sen (%)</th>
<th>Spec (%)</th>
<th>CV</th>
<th>HbA1c (%)</th>
<th>Sen (%)</th>
<th>Spec (%)</th>
<th>CV</th>
<th>FPG (mmol/l)</th>
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<tr>
<td>≥3.9</td>
<td>100</td>
<td>0.22</td>
<td>1</td>
<td>≥4.0</td>
<td>100</td>
<td>0.2</td>
<td>1</td>
<td>≥3.0</td>
<td>100</td>
<td>0</td>
<td>1.0</td>
</tr>
<tr>
<td>≥4.7</td>
<td>100</td>
<td>10.0</td>
<td>1.3</td>
<td>≥5.0</td>
<td>100</td>
<td>11.1</td>
<td>1.3</td>
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Sen, sensitivity; Spec, specificity
increased risk of microvascular complications of type 2 diabetes. Gabir et al. (23) have shown that retinopathy and nephropathy increase at an FPG level of 6.0 mmol/l. Accordingly, we propose that there is a rational basis for using either FPG or HbA1c for purposes of screening and assigning risk and, therefore, targeting the most appropriate group of individuals for further investigation and intervention.

If an FPG level $\geq 7$ mmol/l is used, the incidence of type 2 diabetes is underestimated, as compared with the OGTT (1,8,9,24). Only 4.0% of our subjects were diabetic using FPG based on the ADA criteria, compared with 10.4% using WHO criteria. The detection of type 2 diabetes has been reported by others to halve when ADA as opposed to WHO criteria are applied (1). Wahl et al. (1) showed that at an FPG level of 6.38 mmol/l, the prevalence of diabetes by ADA and 1985 WHO criteria were similar. In the DECODE study, the FPG level that predicted a 2-h value of 11.1 mmol/l was 6.4 mmol/l in men (25). Taken together, the data suggest that an FPG level $\geq 6.4$ mmol/l is the most appropriate level at which to diagnose type 2 diabetes and only 40% (rather than $\sim 60$%) of individuals with type 2 diabetes would be missed. Our study has also shown that when the FPG level is $< 4.7$ mmol/l, type 2 diabetes can be confidently excluded; $\sim 73$% of all subjects had an FPG level between 4.7 and 6.3 mmol/l, and $\sim 17$% of these individuals had an FPG level of 5.6–6.3 mmol/l. Of this latter group, 13.6% (12 of 88) were diabetic based on an OGTT.

The relative cardiovascular risk was at least 2 in the group with FPG $> 6.3$ mmol/l and was 1.7–1.9 in those with an FPG of 5.6–6.3 mmol/l. Therefore, although an FPG of 5.6–6.3 mmol/l is associated with a low risk of microvascular disease, there is a relatively high risk of macrovascular disease. Aggressive treatment of cardiovascular risk factors and attention to lifestyle issues such as diet and exercise are indicated. Moreover, we would argue that lifestyle interventions to prevent progression to type 2 diabetes would be most appropriately targeted to this group (26).

Our data also show that type 2 diabetes is present at an HbA1c (HPLC) $\geq 6.2$%, and the detection rate is better than when the ADA criteria are used. If an OGTT was performed, however, 57% more of the individuals would have been diagnosed as having type 2 diabetes. An HbA1c (HPLC) $< 4.7$% excludes type 2 diabetes. However, $\sim 86$% of all subjects had an HbA1c between 4.7 and 6.1%; 21% of these subjects had an HbA1c of 5.6–6.1%. Of the latter subjects, 21.5% (23 of 107) were diabetic on the OGTT.

We did not observe an independent relationship between HbA1c and cardiovascular risk, although such an association has previously been reported in a larger study (20). The relative cardiovascular risk in our group with an HbA1c (HPLC) $> 6.1$% was at least 2.3, and in those with an HbA1c of 5.6–6.1%, the relative cardiovascular risk was 1.8–2.2. As with FPG, the risk of microvascular disease is low with an HbA1c $< 6.1$%, but a relatively high risk of macrovascular disease remains and accordingly aggressive risk factor reduction is warranted. The situation with HbA1c as measured using DCA2000 is analogous, although the cutoffs were higher. Type 2 diabetes is diagnosed at HbA1c $\geq 6.8$%, whereas HbA1c $< 5.0$% excludes diabetes confidently. The high cardiovascular risk group were those subjects with an HbA1c of 5.8–6.7%.

Wiener et al. (12) found that HbA1c $> 6.2$% had 100% specificity for diagnosing type 2 diabetes. They argued that HbA1c should be used in screening to reduce the number of OGTTs performed but that the OGTT should continue to be used to avoid missing subjects with type 2 diabetes. Interestingly, they acknowledged that if there were a relationship between HbA1c and complications of type 2 diabetes, then it could replace the OGTT. Davidson et al. (27) suggested measuring HbA1c to determine whether diabetes was present in subjects with borderline FPG (6.1–7.7 mmol/l) to avoid inappropriate diagnosis of type 2 diabetes. In the study by Perry et al. (28), an HbA1c $> 6.1$% was considered specific for type 2 diabetes and was used to confirm the presence or absence of the disease in subjects with an FPG level between 5.5 and 8 mmol/l.

We have shown that a reduction in the FPG level at which type 2 diabetes is diagnosed will lead to a comparable increase in the detection rate of diabetes. If we combined FPG and HbA1c, we detected an additional two subjects with diabetes ($\sim 4$%). We would argue, however, that intervention is more logically based on the risk of complications or associated diseases rather than a comparison with arbitrary cutoffs on the OGTT. In a large meta-analysis, HbA1c was proposed as a means to identify diabetic subjects requiring pharmacological intervention (3). There is a relationship between HbA1c and FPG and the risk of both microvascular (4) and macrovascular disease (1,20), although the increased risk of macrovascular disease occurs at lower glycemic thresholds.

In contrast to other studies that have evaluated the use of glucose and HbA1c as screening tests, we studied smaller numbers of subjects prospectively rather than derived data retrospectively but obtained consistent results. Moreover, although we
used self-reported data to evaluate cardiovascular risk, our data relating to cardiovascular risk are consistent with the results of other studies demonstrating an association between cardiovascular disease and increasing FPG and HbA1c, even in the nondiabetic range (18,20,29).

The OGTT is a time-consuming, poorly reproducible, inconvenient, and expensive test that we would argue can largely be avoided in favor of an HbA1c or FPG, using lower diagnostic thresholds and risk factor assessment to provide the most rational approach to subsequent management. We have proposed an algorithm for how this may occur (Fig. 2). Using this algorithm, whether with HbA1c or with FPG, the population is divided into four subsets. The first subset should be considered diabetic because they are at increased risk for cardiovascular and microvascular complications; these subjects should receive standard diabetic assessment (e.g., for retinopathy, neuropathy, and nephropathy) as well as for cardiovascular disease. The second subset is at high cardiovascular risk and at greatest risk for future progression to diabetes. Clinicians should target the cardiovascular risk factors of these subjects and screen for diabetes with HbA1c or FPG every 12–24 months, depending on previous results. The third subset has a cardiovascular risk above baseline although lower results. The third subset has a cardiovascular risk for future progression to diabetes. The fourth subset is at lowest risk and can be reassured, although it is always appropriate to encourage healthy behaviors. The algorithm in Fig. 2 illustrates this approach using FPG and HbA1c (HPLC).

Portable devices for measuring HbA1c are suitable for transport to community settings and, accordingly, provide the potential for mass screening. Fasting is not required and there is no need for further HbA1c measurement in those subsequently determined to have type 2 diabetes. In situations in which fasting blood glucose can be readily obtained, a cutoff of 6.4 mmol/l results in diagnosis of more diabetic subjects than HbA1c as well as identification of those at significant risk for cardiovascular disease, in whom maximal intervention, whether pharmacological or nonpharmacological, should be targeted.

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References


