THE RELATIONSHIP BETWEEN HbA1c AND GLUCOSE LEVELS IN THE GENERAL DUTCH POPULATION: THE NEW HOORN STUDY

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**Objective:** To investigate the relationship between HbA1c, fasting plasma glucose (FPG) and 2 hour postload plasma glucose (2hrPG) in the Dutch general population and to evaluate the results of using HbA1c for screening and diagnosis of diabetes.

**Research Design and Methods:** In 2006/7, 2753 participants of the New Hoorn Study, 40-65 years old who were randomly selected from the population of Hoorn, The Netherlands, underwent an oral glucose tolerance test (OGTT). Glucose status (normal glucose metabolism (NGM), intermediate hyperglycemia (IH), newly diagnosed diabetes (NDM) and known diabetes (KDM)) was defined by the WHO’06 criteria. Spearman correlations were used to investigate the agreement between markers of hyperglycemia and a receiver operating characteristic (ROC) curve was calculated to evaluate the use of HbA1c to identify NDM.

**Results:** In the total population, the correlation between FPG and HbA1c and between 2hrPG and HbA1c were 0.46 and 0.33 respectively. In patients with KDM, these correlations were 0.71 and 0.79. An HbA1c level of ≥ 5.8%, representing 12% of the population, had the highest combination of sensitivity (72%) and specificity (91%) for identifying NDM. This cut-off point would identify 72% of the patients with NDM and include 30% of the individuals with IH.

**Conclusions:** In patients with KDM correlations between glucose and HbA1c are strong, however, moderate correlations were found in the general population. In addition, based on the diagnostic properties of HbA1c defined by ROC curve analysis, the advantage of HbA1c compared to OGTT for the diagnosis of diabetes is limited.
Fasting glucose levels and glucose levels 2-hours after a glucose tolerance test (postload glucose levels) are used for diagnosis and management of diabetes (1). In addition, glycated haemoglobin (HbA1c) levels are used to monitor glycemia in patients with diabetes because it has less day-to-day variability than glucose levels, and is thought to reflect chronic glycemia (2).

In 2007, a consensus statement reported on the worldwide standardization of the HbA1c measurement (3). One of the conclusions was that glycemic goals in clinical practice should be expressed in three types of units, of which one is the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) standardized method. This implies that the unit of measurement of HbA1c will change from % to mmol/mol. The introduction of this new unit of measurement can be confusing for patients and health care providers. Therefore, the ‘A1c-derived average glucose (ADAG)’ study group investigated whether HbA1c can be translated into average blood glucose levels in patients with diabetes (4). A major advantage of using these average glucose levels for chronic glycemia, is that it has the same unit of measurement (mmol/l) as acute glycemia. The ADAG Study Group concluded that average glucose was strongly correlated with HbA1c and that the translation of HbA1c into average glucose levels was therefore possible (4). Moreover, Saudek et al. recommended 1) the use of an HbA1c level of 6.0% or higher as a screening standard for the detection of individuals at high risk of developing diabetes, 2) an HbA1c level of 6.5% confirmed by a glucose test (fasting or oral glucose tolerance test) for the diagnosis of diabetes and 3) HbA1c levels of 7.0% or higher measured twice or confirmed by a glucose test for the diagnosis of diabetes (5). During the review process of the present manuscript, a consensus statement from an International Expert Committee was published that recommended the use of HbA1c levels ≥ 6.5% for the diagnosis of diabetes instead of glucose measures. HbA1c levels between 6.0% and 6.5% are proposed for identifying individuals at high risk of developing diabetes (6). It may however be questioned whether HbA1c is a good indicator of glucose in people with normal or moderately elevated glucose levels and whether it can therefore be used to identify people with intermediate hyperglycemia or undiagnosed diabetes. Therefore, our aim was to investigate the relationship between glucose and HbA1c in the general population and to evaluate the use of HbA1c for the screening and diagnosis of diabetes.

RESEARCH DESIGN AND METHODS

Study design. From July 2006 until November 2007, a population-based study on glucose tolerance was performed in the city of Hoorn, the Netherlands (The New Hoorn Study). A random sample of 6180 men and women aged 40-65 years was drawn from the municipal population registry of Hoorn. Following Dutch privacy legislation, all potential participants received a letter on behalf of the municipality, with a description of the study, and the request to return a form with their name, address and telephone number. When the form was not returned within two weeks, a reminder was sent. To increase the participation rate, a local media campaign was started and participants had the ability to visit the Diabetes Research Center on Saturdays. The study was approved by the Medical Ethics Committee of the VU University Medical Center Amsterdam.

Data collection. Prior to their visit, participants received a questionnaire containing information on demographics, lifestyle, medication and (family) history of disease.
Participants were requested to refrain from eating and drinking (except water) from 8 p.m. the night before the visit and from drinking alcohol from 5 p.m. the day before the visit. They were instructed to follow their usual diet the day prior to each visit, and to be consistent in their diet (both in content, and in approximate timing of evening meals and snacks) and physical activities on the pre-visit days. In addition, participants were requested not to smoke on the morning of the visit and not to come by bicycle. Participants who had not been following these instructions were asked to reschedule the visit.

Upon arrival at the Diabetes Research Center written informed consent was obtained. Height and weight were measured without shoes and heavy clothes. Body Mass Index (BMI) was calculated as weight (kg) divided by height (m) square. The waist circumference was measured between the lower rib margin and the spina iliaca anterior superior, and the hip circumference over the maximum of the buttocks. The waist-to-hip ratio (WHR, waist circumference divided by hip circumference) was calculated. Blood pressure was measured 3 times on the right arm after a 10 minute rest period, using a Colin Press BP 8800p Non-Invasive Blood Pressure Monitor (Colin Medical Technology Corporation, USA). Final blood pressure was calculated as the mean of the last 2 measurements. Fasting whole blood glucose from a capillary vein in the finger was determined on the spot using a HemoCue Beta-glucose analyzer (HemoCue). In participants with a fasting whole blood glucose level below 10 mmol/l, a standard 75-g oral glucose tolerance test (OGTT) was performed. Venous blood samples were drawn before and 120 minutes after glucose ingestion.

**Laboratory assays.** All analyses were performed at the clinical chemistry laboratory of the VU University Medical Center Amsterdam. Glucose was measured in venous plasma by the glucose-oxidase method (Glucoquant/hexokinase/G6P-DH; Boehringer-Mannheim, Mannheim, Germany). HbA1c was assessed using a DCCT standardized reversed-phase cation exchange chromatography (HA 8160 analyzer, Menarini, Florence, Italy) The intra-assay coefficient of variation (CV) was 0.65% at a mean of 4.89% and the inter-assay CV 1.55% at a mean of 5.52%. Triglycerides, total and HDL-cholesterol were determined from fasting plasma samples by enzymatic techniques (Boehringer-Mannheim, Mannheim, Germany). LDL-cholesterol was estimated with the Friedewald formula, except in individuals with triglycerides >4.5 mmol/l.

**Statistical analyses.** Based on the results of the OGTT, participants were categorized into 3 groups using the WHO '06 criteria (1): normal glucose metabolism (NGM), intermediate hyperglycemia (IH) or newly detected diabetes mellitus (NDM). In addition, known diabetes mellitus (KDM) was defined by the use of insulin or oral hypoglycaemic agents and self-reported known diabetes. Differences between the subgroups of glucose tolerance were tested using one-way analysis of variance.

Scatter plots with estimated linear or curvilinear relationships were plotted to describe the relation between glucose and HbA1c in the total population. In addition, the correlations between HbA1c, fasting plasma glucose (FPG) and 2hr postload plasma glucose (2hrPG) were determined using Spearman correlations. After exclusion of participants with KDM, the diagnostic property of HbA1c for the diagnosis of diabetes was evaluated by calculating a receiver operating characteristic (ROC) curve. The area under the ROC curve (AUC) with 95% confidence interval (CI) was calculated. To describe the effect of different cut-off points of HbA1c, sensitivity and specificity
were used. In addition, the positive predictive value (PPV) and negative predictive value (NPV) for every cut-off point were calculated. All analyses were performed using the Statistical Package for the Social Sciences (SPSS for Windows 15.0). The p-value for statistical significance was set at \( \leq 0.05 \).

RESULTS

Population characteristics. Of the 6180 people who were invited, 2807 agreed to participate (45.4%). Of the non-attendees, 47% provided a reason for not participating, of which the most common were: no time to participate (43%) and already having regular health checks (24.5%). After exclusion of 54 participants with missing glucose values, 2753 participants were included for the analysis. Table 1 presents the population characteristics in subgroups with different glucose tolerance status. The prevalences of NGM, IH, NDM and KDM were 77%, 16%, 4% and 3% respectively. Compared to the other categories, participants with KDM were more often male, were older and had a higher mean blood glucose, HbA1c, BMI and WHR. Their blood pressure and lipid levels were lower than in NDM patients, probably due to the treatment regime for diabetes patients used by general practitioners in the Netherlands (7).

Correlations between HbA1c, FPG and 2hrPG. Figure 1 presents the scatter plots for the association between FPG and HbA1c and between 2hrPG and HbA1c. Using a linear association model, R square values, a measure of explained variance, were 0.57 for the association between FPG and HbA1c and 0.35 between 2hrPG and HbA1c (both \( P<0.01 \)). Using exponential or quadratic models did not improve the fit of the model (data not shown), nor could a threshold be identified. In Table 1, the Spearman correlations between HbA1c, FPG and 2hrPG are presented. In the total population, the correlations between HbA1c and FPG were stronger than between HbA1c and 2hrPG (0.46 and 0.33 respectively). Because earlier research provided correlations in subgroups of glucose tolerance status, we also provide correlations within these subgroups (Table 1, bottom rows). Correlations between HbA1c and glucose in subgroups with NGM, IH and NDM ranged between -0.050 and 0.53. In patients with KDM, the correlations were stronger and reached levels between 0.71 and 0.79.

Diagnostic properties of HbA1c. Figure 2 shows the results of the analyses of HbA1c as a possible tool for the screening and diagnosis of diabetes, based on a ROC curve. The AUC was 0.895 (95%CI 0.861-0.930). In Table 2, the diagnostic properties of different HbA1c cut-off points are described. The cut-off point for identifying NDM with the highest sum of sensitivity (72%) and specificity (91%) was an HbA1c level of 5.8%. Of all individuals with an HbA1c level above 5.8%, 24% had diabetic glucose levels. When using the recently proposed cut-off point of 6.0% for the screening of individuals at high-risk of diabetes (5;6), the sensitivity and specificity were 56% and 97% respectively. Less than half of the individuals with HbA1c levels above 6.0% had diabetic glucose levels (PPV of 42%) and 15% of the participants with IH had HbA1c levels above 6.0%. For the diagnosis of diabetes, HbA1c levels above 6.5% and 7.0% were proposed (5;6). Of the participants with HbA1c above 6.5% and 7.0%, 93% and 100% had diabetic glucose levels respectively. However, of all patients with diabetes, 24% had HbA1c levels above 6.5% and 12% had HbA1c levels above 7.0%.

In clinical care, FPG instead of an OGTT is mostly used to diagnose diabetes. To compare the diagnostic properties of HbA1c with FPG, we performed similar ROC curve analyses for FPG as for HbA1c, using OGTT as the criterion. The AUC for FPG was 0.937 (95%CI 0.905-0.969) (data not shown).
DISCUSSION

In the general Dutch population, the correlations between glucose (fasting and postload) and HbA1c were moderate as compared to these correlations in patients with KDM. In addition, when using HbA1c levels \( \geq 6.0\% \) to screen for diabetes in the general population, almost half of the people with diabetic glucose levels would be missed. Last, the HbA1c cut-off point of 7.0\% has a specificity of 100\% and is present among 12\% of the NDM patients.

Nathan et al. recently reported that HbA1c and average glucose levels measured during the day were highly associated in a population of mainly type 1 diabetes patients (4). This is consistent with our findings in patients with diagnosed type 2 diabetes and earlier research (8). Our research however, shows that the correlations between glucose and HbA1c were moderate in a random sample of the general population.

The cut-off point for screening for diabetes with the highest sum of sensitivity and specificity in our data was an HbA1c level of 5.8\%. This cut-off point would detect 72\% of the NDM patients and about 30\% of the individuals at high risk of developing diabetes (participants with IH). Using HbA1c levels of 6.0\% for the screening of individuals at high risk of developing diabetes would lead to identifying 15\% of the individuals with IH. Moreover, 44\% of the NDM patients had HbA1c levels below 6.0\%. This is consistent with the findings in the NHANES study, in which an HbA1c level of 5.8\% was also found to have the highest sum of sensitivity and specificity (9;10) and in which 60\% of the NDM patients had HbA1c levels below 6.1\% (11).

Since all participants with HbA1c levels above 7.0\% had diabetic glucose levels, our data confirm that HbA1c levels of 7.0\% or above can be used as a threshold not requiring further glucose testing for the diagnosis of diabetes. A limitation of this diagnostic criterion is the low sensitivity as shown in our study as well as in the NHANES studies (9;10). Therefore, in most of the people, the OGTT would still be necessary to verify the diagnosis. Moreover, our data showed that FPG had a higher area under the ROC curve than HbA1c, indicating a higher diagnostic value of FPG than HbA1c for identifying NDM. Other population-based research concluded that HbA1c had no additional diagnostic value compared to fasting glucose alone (12), except in high-risk groups (13). Despite the presently discussed limitations of HbA1c for the diagnosis of diabetes compared to an OGTT, HbA1c might be superior compared to OGTT in terms of cost-effectiveness and practical use in the clinical setting, since fasting measures are not necessary and it is less time consuming than an OGTT. Moreover, HbA1c might be a good indicator for future complications. Large scale epidemiological studies have found that HbA1c is associated with complications like cardiovascular disease, even in the non-diabetic range of glucose tolerance (14;15).

The moderate correlations of glucose with HbA1c found in this study, may imply that HbA1c and glucose reflect different processes, especially in the non-diabetic range of glucose tolerance. The amount of glycosylation is known to vary inter-individually (16;17). Possible mechanisms are genetic (18), aging (19), differences in erythrocyte environments (20), heterogeneity in red cell lifespan (21) and the role of racial differences (22). In participants with KDM, the correlations between glucose and HbA1c were stronger than in participants with NDM. It can be speculated that a longer diabetes duration is accompanied with changes in the intra-erythrocyte environment. Another explanation may be found in the degree of glycemic control. Kilpatrick et al. showed in the DCCT trial that for any given HbA1c, the plasma glucose levels of participants in
different treatment groups were not the same (23).

The New Hoorn Study is a population study of individuals who were randomly selected. Therefore, we had the ability to study the relation between HbA1c and glucose in a representative sample of the Dutch population with different glucose tolerance stadia. A limitation of the present study was that 45.4% of the originally invited sample agreed to participate. As a result, we cannot exclude the possibility that self-selection occurred, possibly leading to over-representation of individuals with an interest in diabetes. Second, we did not have the availability of continuous glucose monitoring during the day and only one OGTT was performed on all participants. It can be suggested that the moderate correlations between glucose and HbA1c in the present study, are due to the rather high variability of FPG and 2hrPG within individuals (24). However, de Vegt et al. showed that this variability did not result in a different reproducibility for the categorization of glucose tolerance over 6 years (25). In addition, the correlations between glucose and HbA1c in patients with KDM found in our study are comparable to the correlations found in the study by Nathan et al. in which continuous glucose monitoring was used in known diabetes patients (4). Correlation coefficients are sensitive for the differences in ranges of the included variables and tend to be lower in subgroups with a smaller range. The range of glucose and HbA1c is large in patients with diabetes, resulting in strong correlations. However, in our study the correlations between glucose and HbA1c were stronger in participants with diabetes compared to the total population, while the ranges of both glucose and HbA1c are larger in the total population. This indicates that differences in ranges are not the only explanation of the differences found in the correlations between the total population and subpopulations with diabetes.

To conclude, our results support the ongoing research on the translation of HbA1c into HbA1c derived glucose in people with KDM. However, based on the moderate correlations between glucose and HbA1c in the total sample, translation of HbA1c into average glucose in the general population is not recommendable. Moreover, based on diagnostic properties only, the advantage of using HbA1c instead of an OGTT for both screening and diagnosis of diabetes is limited, while glucose measures will still be necessary in most cases to verify the outcome. However, research including cost-effectiveness, practical use and the role of HbA1c in the development of future complications is needed to further explore this.

**Disclosure.** No potential conflicts of interest relevant to this article were reported.

Figure Legends:

Figure 1. Scatter plots of fasting plasma glucose and 2hr postload plasma glucose in relation to HbA1c in the total population. Diabetes patients are indicated by dotted reference lines at fasting plasma glucose levels of 7.0 mmol/l and 2hr postload plasma glucose levels of 11.1 mmol/l.

Figure 2. Receiver-operating characteristic curves for identification of participants with previously undiagnosed diabetes, using HbA1c for diagnosis and an OGTT as criterion. HbA1c cut-off points of 5.8%, 6.0%, 6.5% and 7.0% are indicated in the curve.
REFERENCES
Table 1. Population characteristics and Spearman correlations between markers of hyperglycemia in the total population and in participants with NGT, IH, NDM or KDM.

<table>
<thead>
<tr>
<th></th>
<th>Total population</th>
<th>NGM</th>
<th>IH</th>
<th>NDM</th>
<th>KDM</th>
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<tr>
<td>N (%)</td>
<td>2753</td>
<td>2122 (77)</td>
<td>439 (16)</td>
<td>107 (4)</td>
<td>85 (3)</td>
</tr>
<tr>
<td>Male (%)</td>
<td>46.9</td>
<td>44.2</td>
<td>56.4</td>
<td>47.2</td>
<td>64.6 * *, †</td>
</tr>
<tr>
<td>Age (years)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>7.3 (8.14)</td>
</tr>
<tr>
<td>Not of Dutch origin (%)</td>
<td>10.8</td>
<td>9.8</td>
<td>13.2 *</td>
<td>17.6 *</td>
<td>13.9</td>
</tr>
<tr>
<td>DM duration (years)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHG)</td>
<td>133.3 (18.1)</td>
<td>130.8 (17.4)</td>
<td>140.9 (18.2) *</td>
<td>146 (20.5) * *, †</td>
<td>138.6 (17.2) * *, †</td>
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<td>Diastolic blood pressure (mmHg)</td>
<td>76.6 (10.6)</td>
<td>75.5 (10.4)</td>
<td>80.2 (10.4)</td>
<td>82.6 (10.5)</td>
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<td>Weight (kg)</td>
<td>79.0 (14.6)</td>
<td>77.2 (13.5)</td>
<td>84.1 (15.9) *</td>
<td>86.1 (15.4) *</td>
<td>91.0 (17.2) * *, †</td>
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<td>BMI (kg/m²)</td>
<td>26.3 (4.0)</td>
<td>25.6 (3.7)</td>
<td>27.9 (4.2)</td>
<td>28.8 (4.3)</td>
<td>30.0 (5.1) * *, †</td>
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<tr>
<td>Waist (cm)</td>
<td>89.8 (11.8)</td>
<td>87.7 (10.7)</td>
<td>95.3 (11.7)</td>
<td>99.1 (11.7) * , †</td>
<td>103.1 (15.5) * *, †</td>
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<td>Waist-to-hip ratio</td>
<td>0.89 (0.08)</td>
<td>0.88 (0.08)</td>
<td>0.93 (0.08)</td>
<td>0.95 (0.07)</td>
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<td>Total cholesterol (mmol/l)</td>
<td>5.5 (1.0)</td>
<td>5.4 (0.96)</td>
<td>5.7 (1.07)</td>
<td>5.7 (1.08)</td>
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<td>HDL cholesterol (mmol/l)</td>
<td>1.51 (0.42)</td>
<td>1.56 (0.42)</td>
<td>1.38 (0.39)</td>
<td>1.28 (0.38)</td>
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<td>Triglycerides § (mmol/l)</td>
<td>1.5 (1.1)</td>
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<td>Fasting plasma glucose (mmol/l)</td>
<td>5.6 (0.95)</td>
<td>5.30 (0.38)</td>
<td>6.0 (0.51)</td>
<td>7.7 (2.24) * , †</td>
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<td>2hr postload plasma glucose (mmol/l)</td>
<td>5.9 (2.4)</td>
<td>5.09 (1.13)</td>
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<td>11.50 (3.24) * , †</td>
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<td>HbA1c (%)</td>
<td>5.5 (0.52)</td>
<td>5.3 (0.26)</td>
<td>5.6 (0.33) *</td>
<td>6.4 (1.25) * , †</td>
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<td>HbA1c vs. fasting plasma glucose ‡</td>
<td>0.46 * *</td>
<td>0.26 *</td>
<td>0.25 *</td>
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<td>0.66 *</td>
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Values are mean ± SD, percentage or median (25,75 percentile)
*Significantly different from NGT
†Significantly different from IH
‡Significantly different from NDM
§ log-transformed before testing
‖ Spearman correlations
* Statistically significant (p ≤ 0.05) correlation

<table>
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<tr>
<th>HbA1c (%)</th>
<th>% of total population</th>
<th>% of high risk (IH)</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
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<th>NPV (%)</th>
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Figure 1.
Figure 2.