Original Paper

# Comparison of Hemoglobin $A_{1c}$ and Fasting Glucose Criteria to Diagnose Diabetes Among People With Metabolic Syndrome and Fasting Glucose Above 100 mg/dL (5.5 mmol/L)

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*The aim of this study was to compare hemoglobin $A_{1c}$ ($HbA_{1c}$) and fasting glucose for the diagnosis of diabetes among people with metabolic syndrome and fasting glucose >100 mg/dL (5.5 mmol/L). Consecutive individuals (N=142) with metabolic syndrome and fasting glucose >100 mg/dL (5.5 mmol/L) but without a self-reported history of diabetes who visited the outpatient lipid and obesity clinic of the University Hospital of Ioannina, Greece from January through September 2009 were included. $HbA_{1c}$ ≥6.5% and fasting glucose ≥126 mg/dL (7 mmol/L) were used separately to define diabetes. Overall, 29.5% of patients had both $HbA_{1c}$ ≥6.5% and fasting glucose ≥126 mg/dL (7 mmol/L), 25.3% had $HbA_{1c}$ ≥6.5% but fasting glucose <126 mg/dL (7 mmol/L), and 9.1% had $HbA_{1c}$ <6.5% but fasting glucose ≥126 mg/dL (7 mmol/L). A greater proportion of patients reached a diagnosis of diabetes based on the $HbA_{1c}$ criterion (n=78, 54.9%) compared with the fasting glucose criterion (n=55, 38.7%, P=.000). A large proportion of patients (44.8%) with impaired fasting glucose (fasting glucose 100–125 mg/dL; 5.6–6.9 mmol/L) would be classified as diabetics using the $HbA_{1c}$ criterion. Implication of the $HbA_{1c}$ criterion may increase the rate of diabetes diagnosis among people with metabolic syndrome and fasting glucose >100 mg/dL (5.5 mmol/L).* J Clin Hypertens (Greenwich). *2010;12:543–548.* ©*2010 Wiley Periodicals, Inc.*

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Traditionally, diabetes was defined by using a fasting plasma glucose (FPG) ≥126 mg/dL (7.0 mmol/L). In June 2009, the International Expert Committee released a report which recommended the use of hemoglobin $A_{1c}$ ($HbA_{1c}$) ≥6.5% to diagnose diabetes.[1] This recommendation was subsequently adopted by the American Diabetes Association (ADA)[2] in its 2010 report on the classification and diagnosis of diabetes. They also proposed that individuals with $HbA_{1c}$ values of 5.7% to 6.4% are at high risk for diabetes.[2] Previously, $HbA_{1c}$ had been used primarily to monitor glycemic control among persons with diabetes. However, over the last decade, the

$HbA_{1c}$ measurement has become standardized facilitating its recognition as an acceptable diagnostic method for diabetes.[3,4]

The prevalence of diabetes in some populations may not be the same when diagnosis is based on $HbA_{1c}$ compared with diagnosis with fasting glucose, and one method may identify different individuals than the other as measurements of glucose levels and $HbA_{1c}$ reflect different aspects of glucose metabolism. In a recent comparison of $HbA_{1c}$ and fasting glucose criteria to diagnose diabetes among 6890 US adults who participated in the 1999–2006 National Health and Nutrition Examination Survey (NHANES), the $HbA_{1c}$ criterion demonstrated reasonable agreement with fasting glucose.[5] However, it is not known if this is also the case in selected populations at high risk for diabetes.

The purpose of this study was to compare $HbA_{1c}$ $\geq$6.5% and FPG $\geq$126 mg/dL (7 mmol/L) for the identification of undiagnosed diabetes among people with metabolic syndrome and fasting glucose >100 mg/dL (5.5 mmol/L) who visited the outpatient lipid and obesity clinic of our hospital. Additionally, we evaluated the demographic characteristics and cardiovascular risk profile for individuals diagnosed with diabetes by each of these methods.

## METHODS

### Participants

Consecutive individuals with metabolic syndrome and fasting glucose >100 mg/dL (5.5 mmol/L) but without a self-reported history of diabetes who visited the outpatient lipid and obesity clinic of the University Hospital of Ioannina, Greece from January through September 2009 were included in the present study. Metabolic syndrome was diagnosed by the National Heart, Lung, and Blood Institute and the American Heart Association (NHLBI/AHA) definition.[6] Specifically, the definition of metabolic syndrome was established with the presence of $\geq$3 of the following criteria: waist circumference >88 cm in women or >102 cm in men, FPG $\geq$100 mg/dL (5.5 mmol/L), blood pressure >130/85 mm Hg or treatment with antihypertensive drugs, high-density lipoprotein cholesterol (HDL-C) <50 mg/dL (1.3 mmol/L) in women or <40 mg/dL (1.0 mmol/L) in men or treatment with HDL-C–raising drugs, and triglycerides >150 mg/dL (1.7 mmol/L) or treatment with triglyceride-lowering medications.[6] All study participants gave their written informed consent and the Ethics Committee of the University Hospital of Ioannina approved the study protocol.

### Measurements

All laboratory measurements were carried out after an overnight fast. Plasma glucose was measured using a modified hexokinase enzymatic method. The determination of $HbA_{1c}$ was based on a standardized latex agglutination inhibition assay (Randox Laboratories Ltd., Antrim, UK). $HbA_{1c}$ values are expressed as percentage of the total hemoglobin concentration. The sensitivity of the method is 0.25 g/dL of $HbA_{1c}$ and the within run and between run precision <6.67% and <4.82%, respectively. Fasting serum insulin levels were measured by an AxSYM insulin assay microparticle enzyme immunoassay on an AzSYM analyzer (Abbott Diagnostics, Chicago, IL). The homeostasis model assessment Insulin Resistance (HOMA-IR) index was calculated as follows: HOMA-IR index = fasting insulin (mU/L) $\times$ fasting glucose (mg/dL)/405.[7]

Serum total cholesterol, HDL-C and triglycerides, uric acid, creatinine, total bilirubin, as well as serum activities of aspartate aminotransferase, alanine aminotransferase, and gamma-glutamyl transpeptidase were determined enzymatically in the laboratory of the University Hospital of Ioannina using an Olympus AU 600 analyzer (Olympus Diagnostica GmbH, Hamburg, Germany). Low-density lipoprotein cholesterol was calculated using the Friedewald formula (provided that triglycerides were <400 mg/dL; 4.5 mmol/L). Estimated glomerular filtration rate was estimated using the Modification of Diet in Renal Disease (MDRD) study equation.[8]

### Statistics

Values are given as mean $\pm$ standard deviation and median (range) for parametric and nonparametric data, respectively. The chi-square test was used to compare categorical variables. Continuous variables were tested for lack of normality by the Kolmogorov-Smirnov test and logarithmic transformations were accordingly performed for nonparametric variables. One-way analysis of variance was used for comparisons between groups followed by the least significant differences test (in case of significant effects) for multiple pairwise comparisons, except for serum triglycerides, insulin and HOMA index, where the Kruskal-Wallis analysis of variance median test was used followed by the Mann-Whitney U test for pairwise comparisons because of their skewed distribution. Correlations between parameters were evaluated using Pearson or Spearman correlation coefficients as appropriate.

Significance was defined as $P<.05$. Analyses were performed using the Statistical Package for

the Social Sciences (SPSS) 15.0 (SPSS Inc, Chicago, IL).

## RESULTS

One hundred forty-two individuals (73 males, mean age 62 years old) were included in the present study. Characteristics of study participants are shown in Table I. By inclusion criteria all participants had metabolic syndrome and a fasting glucose >100 mg/dL (5.5 mmol/L).

$HbA_{1c}$ and fasting glucose were highly correlated in the entire cohort ($r$=0.698, $P$=.000) (Figure 1). Overall, 29.5% (n=42) of patients had both $HbA_{1c}$ ≥6.5% and fasting glucose ≥126 mg/dL (7 mmol/L), 25.3% (n=36) had $HbA_{1c}$ ≥6.5% but fasting glucose <126 mg/dL (7 mmol/L), and 9.1% (n=13) had $HbA_{1c}$ <6.5% but fasting glucose ≥126 mg/dL (7 mmol/L) (Figure 2). FPG and $HbA_{1c}$ measurements were repeated (a few weeks apart) for confirmation in a subgroup of participants (n=49). We observed a very good agreement for both FPG ($r$=0.77, $P$<.00) and $HbA_{1c}$ values ($r$=0.80, $P$<.00). In only 2 cases there was a disagreement in participant classification based on FPG results; the first measurement was taken into account in these 2 patients.

A significantly greater proportion of patients reached a diagnosis of diabetes based on the $HbA_{1c}$ criterion (n=78, 54.9%) compared with the fasting glucose criterion (n=55, 38.7%, $P$=.000). Table II shows the demographic and clinical characteristics of participants according to criteria fulfilled for the diagnosis of diabetes. Participants who had both criteria exhibited significantly higher fasting glucose and $HbA_{1c}$ values compared with the other 2 groups. No other significant difference between groups was found. Use of blood pressure and lipid lowering drugs also did not differ between groups (data not shown).

Eighty seven participants (61.3%) had impaired fasting glucose (IFG; fasting glucose 100–125 mg/dL; 5.6–6.9 mmol/L). A large proportion of these participants (n=39, 44.8%) would be classified as diabetics using the $HbA_{1c}$ criterion ($HbA_{1c}$ = 6.9%±0.3%). Additionally, 32 IFG participants (36.7%) had an $HbA_{1c}$ between 5.7% and 6.4% and 16 (18.4%) had an $HbA_{1c}$ of <5.7%.

**Table I.** Characteristics of Study Participants (N=142)

| | |
|---|---|
| Age | 62±10 |
| Sex, male/female | 73/69 |
| Smoking, yes/no | 41/101 |
| Weight, kg | 81.7±16.2 |
| BMI, kg/m$^2$ | 29.9±5.3 |
| Waist circumference, cm | 103.4±12.8 |
| Family history of diabetes, % | 19 |
| Systolic blood pressure, mm Hg | 137±9 |
| Diastolic blood pressure, mm Hg | 85±5 |
| Hypertension, % | 83.8 |
| ARBs, % | 50.8 |
| ACEIs, % | 8.5 |
| CCBs, % | 28.2 |
| BBs, % | 22.5 |
| HCTZ, % | 35.3 |
| Statin, % | 57.7 |
| Fibrate, % | 16.6 |
| Other LLDs, % | 7.8 |

Abbreviations: ACEIs, angiotensin converting enzyme inhibitors; ARBs, angiotensin receptor blockers; BBs, β-blockers; BMI, body mass index; CCBs, calcium channel blockers; HCTZ, hydrochlorothiazide; LLDs, lipid-lowering drugs.

## DISCUSSION

This study compared the new recommendation by the International Expert Committee and the ADA to use $HbA_{1c}$ to diagnose diabetes with the fasting glucose criterion among participants at high risk for undiagnosed diabetes. Use of $HbA_{1c}$ significantly and greatly increased the percentage of participants diagnosed with diabetes compared with fasting glucose (54.9% vs 38.7%, respectively). Most of these extra diabetes cases derived from the group of participants with IFG. Indeed, almost half of the IFG participants were diagnosed with diabetes using the $HbA_{1c}$ criterion. These may have been diagnosed by an oral glucose tolerance test (OGTT) which was not available for the majority of participants in this study.

Discordance in the diagnosis of diabetes using $HbA_{1c}$ and fasting glucose is expected and is likely due to the assessment of different aspects of glucose metabolism.[1] In the recent analysis among 6890 US adults who participated in the 1999–2006 NHANES use of $HbA_{1c}$ to diagnose diabetes would result in the same classification as fasting glucose for 97.7% of US adults. For those with discordant results, 0.5% of US adults had $HbA_{1c}$ ≥6.5% and fasting glucose <126 mg/dL (7 mmol/L) whereas 1.8% had $HbA_{1c}$ <6.5% and fasting glucose ≥126 mg/dL (7 mmol/L).[5] These results are quite different compared with those of our study in which only 29.5% of patients had both criteria, 25.3% had $HbA_{1c}$ ≥6.5% but fasting glucose <126 mg/dL (7 mmol/L), and 9.1% had $HbA_{1c}$ <6.5% but fasting glucose ≥126 mg/dL (7 mmol/L). Another analysis of the NHANES data

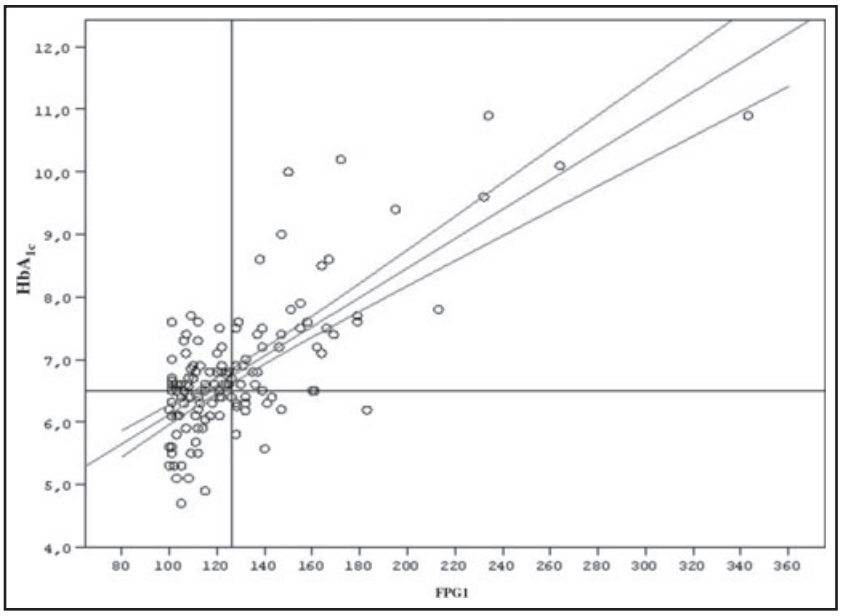


*Figure 1. Correlation between fasting plasma glucose (FPG 1) and hemoglobin $A_{1c}$ ($HbA_{1c}$) levels.*

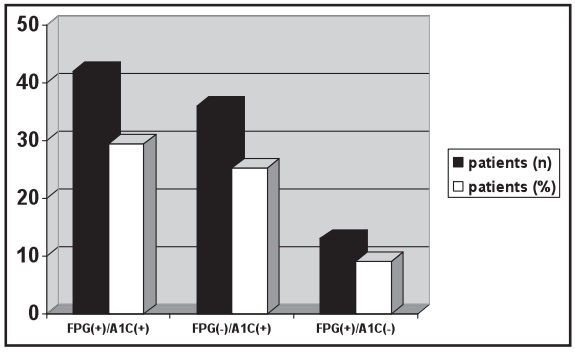


*Figure 2. Classification of participants according to the number of criteria for the diagnosis of diabetes. FPG indicates fasting plasma glucose; $HbA_{1c}$, hemoglobin $A_{1c}$.*

revealed that one-third fewer cases of undiagnosed diabetes were detected using the $HbA_{1c}$ cutpoint of $\geq$6.5% compared with the FPG cutpoint of $\geq$126 mg/dL (7.0 mmol/L).[9] In contrast, more patients reached a diagnosis of diabetes with the $HbA_{1c}$ compared with the FPG criterion in our study. It seems that in the general population these 2 criteria are in good agreement. However, in selected groups, such as in people with metabolic syndrome and a fasting glucose >100 mg/dL (5.5 mmol/L), use of $HbA_{1c}$ recognizes as diabetics a large proportion of individuals who are usually classified as IFG based on fasting glucose. In this context, ADA also stated that "Further research is needed to better characterize those patients whose glycemic status might be categorized differently by two different tests (eg, FPG and $HbA_{1c}$), obtained in close temporal approximation."[2] One key difference in study populations between the current report and the NHANES data is the age of the cohort. In fact, individuals diagnosed as diabetics with the $HbA_{1c}$ compared with the FPG criterion were younger (53.1 vs 60.0 years, $P<.05$) in NHANES.[5] Although similar findings have been previously reported by other investigators,[10,11] no between-group difference in age was noted in our study (mean age 62 years). The choice of the participants (ie, participants at high risk of undiagnosed diabetes) may have eliminated the influence of age observed in the general population.

On the other hand, use of $HbA_{1c}$ would have missed a small proportion (9.1%) of people with $HbA_{1c}$ <6.5% but fasting glucose $\geq$126 mg/dL (7 mmol/L). These people had an $HbA_{1c}$ of 6.2%$\pm$0.2%, ie, they would be characterized as high risk for diabetes by the new recommendation.[2] Although these individuals would not satisfy the new $HbA_{1c}$ recommendation for the diagnosis of diabetes, they would be targeted for preventive therapy to reduce diabetes risk, which may also prompt a fasting glucose measurement. Only 2 out of these 13 participants had an $HbA_{1c}$ <5.7% and would have been totally missed.

As may have been anticipated, people who fulfilled both criteria had greater fasting glucose and $HbA_{1c}$ values compared with those having only one criterion (Table II). This was also the case in the NHANES analysis.[5] No other difference in the

**Table II.** Characteristics of Participants According to Criteria Used for Diabetes Diagnosis

| | FPG(+)/HbA$_{1c}$(+) (N=42) | FPG(−)/HbA$_{1c}$(+) (N=36) | FPG(+)/HbA$_{1c}$(−) (N=13) | *P* Value |
|---|---|---|---|---|
| Age, years | 61.5±12.1 | 64.3±7.9 | 63.5±7.2 | NS |
| Sex, male/female | 23/19 | 22/14 | 8/5 | NS |
| Smoking, yes/no | 16/26 | 14/22 | 3/10 | NS |
| BMI, kg/m$^2$ | 31.3±.3 | 30.5±4.6 | 30.1±3.7 | NS |
| Waist circumference, cm | 105.6±13.8 | 106.3±16.0 | 104.0±6.9 | NS |
| FPG, mg/dL | 158.2±42.9[a,b] | 110.6±8.6 | 138.5±15.0[a] | .000 |
| HbA$_{1c}$, % | 7.7±1.2[a,d] | 6.9±0.4[c] | 6.2±0.2 | .000 |
| T-CHOL, mg/dL | 207±47 | 205±41 | 181±42 | NS |
| TGs, mg/dL | 135 (67–352) | 139 (77–378) | 127 (60–240) | NS |
| HDL-C, mg/dL | 50±16 | 51±11 | 46±13 | NS |
| LDL-C, mg/dL | 125±39 | 124±39 | 107±37 | NS |
| Non–HDL-C, mg/dL | 157±41 | 154±41 | 135±39 | NS |
| γGT, U/L | 22 (10–107) | 23 (11–93) | 23 (12–55) | NS |
| AST, U/L | 23±11 | 23±7 | 23±6 | NS |
| ALT, U/L | 28±18 | 23±9 | 29±19 | NS |
| Insulin, μU/mL | 10.3 (1.0–28.3) | 12.4 (4.4–36.0) | 10.1 (3.7–40.7) | NS |
| HOMA-IR | 3.9 (0.3–15.4) | 3.1 (1.0–9.3) | 3.2 (1.2–14.8) | NS |
| TBIL, mg/dL | 0.76±0.44 | 0.70±0.27 | 1.00±0.55 | NS |
| Uric acid, mg/dL | 5.3±1.3 | 5.9±1.6 | 6.3±2.0 | NS |
| SCr, mg/dL | 0.94±0.17 | 0.99±0.20 | 1.00±0.22 | NS |
| e-GFR, mL/min/1.73 m$^2$ | 76.4±14.3 | 70.1±14.9 | 64.6±12.1 | NS |
| Systolic blood pressure, mm Hg | 137±9 | 138±10 | 135±7 | NS |
| Diastolic blood pressure, mm Hg | 85±6 | 86±4 | 85±4 | NS |
| Hypertension, % | 85 | 88 | 84 | NS |

Abbreviations: HbA$_{1c}$, hemoglobin A$_{1c}$; ALT, alanine aminotransferase; AST, aspartate transaminase; BMI, body mass index; e-GFR, estimated glomerular filtration rate; FPG, fasting plasma glucose; HOMA-IR, homeostasis model assessment insulin resistance; LDL-C, low-density lipoprotein cholesterol, HDL-C, high-density lipoprotein cholesterol; SCr, serum creatinine; TBIL, total bilirubin; T-CHOL, total cholesterol; TGs, triglycerides ; γGT, gamma-glutamyl transpeptidase. To convert values for triglycerides to mmol/L multiply by 0.01129. To convert values for cholesterol to mmol/L multiply by 0.02586. To convert values for glucose to mmol/L multiply by 0.05551. To convert values for creatinine to μmol/L multiply by 88.4. To convert values for uric acid to μmol/L multiply by 59.5. To convert values for bilirubin to μmol/L multiply by 17.1. [a]$P<.0001$ vs FPG(−)/HbA$_{1c}$(+) group. [b]$P<.05$ vs FPG(+)/HbA$_{1c}$(−) group. [c]$P<.01$ vs FPG(+)/HbA$_{1c}$(−) group. [d]$P<.0001$ vs FPG(+)/HbA$_{1c}$(−) group.

clinical or laboratory characteristics was found between the 3 groups.

HbA$_{1c}$ has many advantages over fasting glucose and may become the test of choice for the diagnosis of diabetes worldwide. Specifically, compared with the measurement of glucose, the HbA$_{1c}$ assay is at least as good at defining the level of hyperglycemia at which retinopathy prevalence increases; has appreciably superior technical attributes, including less preanalytic instability and less biologic variability; and is more clinically convenient.[1,2] Importantly, progress in standardization of methods for HbA$_{1c}$ measurement has significantly reduced the variation among them.[12] We should note, however, that there are several limitations in HbA$_{1c}$ measurement. These include high cost, restricted availability of the assay in some regions of the developing world, and incomplete correlation between HbA$_{1c}$ and average glucose in certain individuals.[2] Furthermore, HbA$_{1c}$ is frequently misleading in patients with some types of anemia and hemoglobinopathies; these disorders may also play a role in the differences observed among various ethnic or geographic distributions.[2] In addition, the accuracy of HbA$_{1c}$ measurements partly depends on proper storage and handling (eg, temperature, duration) of samples prior to analysis.[13] Specifically, the ion-exchange methods have been associated with a marked increase in HbA$_{1c}$ values after a few days of storage. In contrast, the colorimetric and affinity methods are superior to ion exchange in situations where long delays between sample collection and assay cannot be avoided.[14] At 4°C all methods seem to give acceptable results for samples stored for as long as a week, while at 20°C the colorimetric and affinity methods showed sample stability for ≥7 days.[13] Under proper conditions, whole blood

can be stored at room temperature up to 21 days before $HbA_{1c}$ measurement both with affinity and "high-performance" liquid ion-exchange chromatography.[15]

The ultimate goal is to identify individuals at risk for diabetes complications so that they can be treated. The $HbA_{1c}$ diagnostic level of 6.5% accomplishes this goal according to the International Expert Committee.[1] Our study adds to this recommendation by showing that $HbA_{1c}$ recognizes as diabetics a large proportion of participants with metabolic syndrome and a fasting glucose >100 mg/dL (5.5 mmol/L) who would not be classified as such based only on fasting glucose. This may lead to the prompt initiation of effective preventive strategies in these individuals, thus resulting in better long-term prognosis.

### Study Limitations

We studied a highly selected group of patients and thus our results may not be extrapolated to other populations. However, studying this population is clinically relevant as it comprises a large group of patients who visit outpatient vascular disease prevention clinics. We did not perform an OGTT in most participants which could have identified patients with IFG who actually had diabetes. However, an OGTT is difficult to do in every day clinical practice. Furthermore, regarding the diagnosis of diabetes, it has been suggested that OGTT identifies about 2% more individuals than does FPG.[16] Also, OGTT has poor reproducibility compared with other glucose-based tests or $HbA_{1c}$.[17]

## CONCLUSIONS

If also confirmed by larger studies, implication of the $HbA_{1c}$ criterion may largely increase the rate of diabetes diagnosis among people with metabolic syndrome and a fasting glucose >100 mg/dL (5.5 mmol/L). This will have important health and economic consequences.

*Disclosures: The authors have no conflicts of interest to report in relation to this study.*

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