Hemoglobin A₁c and Fasting Plasma Glucose Levels as Predictors of Retinopathy at 10 Years

The French DESIR Study

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Objective: To evaluate the predictive values of hemoglobin A₁c (HbA₁c) and fasting plasma glucose (FPG) for retinopathy 10 years after the baseline examination.

Methods: Seven hundred men and women from the DESIR (Data From an Epidemiological Study on the Insulin Resistance Syndrome) Study underwent evaluation for retinopathy using a nonmydriatic digital camera. During the preceding 9 years, 235 had diabetes mellitus (treated or FPG level of ≥126 mg/dL at least once), 227 had an impaired FPG level (110-125 mg/dL) at least once, and 238 always had glucose levels within reference limits (<110 mg/dL).

Results: Compared with those without retinopathy, the 44 participants with retinopathy at 10 years had higher baseline mean (SD) levels of FPG (130 [49] vs 106 [22] mg/dL) and HbA₁c (6.4% [1.6%] vs 5.7% [0.7%]) (both, baseline mean (SD) levels of FPG (130 [49] vs 106 [22] mg/dL). The frequency of retinopathy at 10 years, standardized according to the distribution of glycemia across the entire DESIR population, was 3.6%. In our population, FPG levels of 108 and 116 mg/dL had positive predictive values of 8.4% and 14.0%, respectively, for retinopathy at 10 years; HbA₁c levels of 6.0% and 6.5% had positive predictive values of 6.0% and 14.8%, respectively. After 10 years of follow-up, retinopathy was equally frequent in participants with impaired FPG levels and in those who became diabetic during the study (8.6% and 6.7%, respectively), lower than in those with diabetes at baseline (13.9%).

Conclusion: Because the positive predictive values for retinopathy increase sharply from 108 mg/dL for FPG and from 6.0% for HbA₁c levels, these thresholds are proposed to identify those at risk of retinopathy 10 years later.


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IT IS WELL ACCEPTED THAT THE HYPERglycemia accompanying diabetes mellitus is associated with microvascular complications, in particular retinopathy, and indeed one of the reasons for choosing the thresholds in the current definition of diabetes was that the frequency of retinopathy and nephropathy started to increase in cross-sectional studies above these levels of glucose and hemoglobin A₁c (HbA₁c).1-2 The determination of thresholds has been performed by eye, and in some studies a change-point model3 has been used to identify the glycemic level at which the prevalence of retinopathy changes from a constant to an increasing rate.

However, some controversy concerns the actual value of this glycemic threshold for identifying retinopathy. Wong et al4 reexamined the relationship between fasting plasma glucose (FPG) level and retinopathy in 3 contemporary studies and found that, although the prevalence of retinopathy increased with FPG concentrations, there was no clear diagnostic cutoff. More recently, in Malay adults from Singapore, HbA₁c thresholds of 6.6% and 7.0% (to convert to proportions of total hemoglobin, multiply by 0.01) were identified in relation to mild and moderate retinopathy, respectively, using “optimal values” from receiver operating characteristic (ROC) curves.5 It is now well established that the nondiabetic population also has retinopathy,6-14 albeit at a lower frequency than patients with diabetes and in a milder form, indicating that there may be factors other than FPG levels that increase the risk of retinopathy.

Many more cross-sectional than prospective studies of retinopathy have been performed.4 There are few studies of inci-
dent retinopathy in general populations, and most of these have focused on FPG or HbA1c levels, but rarely both, as risk factors for retinopathy. Some other risk factors for incident retinopathy have been identified.13-18 The Bedford Survey in the United Kingdom was one of the earliest to study the presence of retinopathy at 7 years in individuals with borderline diabetes at baseline, namely, a 2-hour glucose level of 120 to 200 mg/dL (to convert to millimoles per liter, multiply by 0.0555) after a 50-g oral glucose tolerance test.6 One of the most complete studies was conducted among the Pima Indians19 and included almost 1000 individuals who were followed up for 5 years. Dividing the FPG, 2-hour glucose, and HbA1c, levels into deciles, they showed a sharp increase in incident retinopathy in the 2 highest decile groups.

The aim of this report was to study the frequency of retinopathy in individuals 10 years after baseline according to baseline levels of FPG and HbA1c, and to evaluate positive predictive values for retinopathy at various levels of these 2 glycemic variables. The secondary objective was to evaluate potential risk factors associated with retinopathy at baseline and during the 9 years preceding the evaluation of retinopathy.

METHODS

PARTICIPANTS

The men and women in this study participated in the DESIR (Data From an Epidemiological Study on the Insulin Resistance Syndrome) Study cohort and were recruited in 1994 to 1996 from consultants in 10 French Social Security health examination centers in the central western part of France.20 At entry into the study, participants were aged 30 to 65 years, and they were invited to health examinations at 3, 6, and 9 years after inclusion. After the 9-year examination, the 321 individuals who had been treated for diabetes mellitus or who had an FPG level of at least 126 mg/dL at some time during the study were invited to attend a special examination on microvascular complications of diabetes and, in particular, an examination of the retina. Of these, 237 (73.8%) participated, and 17 (5.3%) had died. For comparison, 2 other groups matched by age, sex, and examination center with those in the diabetes group were also invited to the retinal examination. Group 1 had an impaired fasting glucose (IFG) level (ie, FPG level, 110-125 mg/dL) at any time during the study; group 2 had glucose levels within reference limits (FPG level, <110 mg/dL) during the entire study. There were 246 individuals in the IFG group (68.3% participation; 9 had died) and 249 (56.1% participation; 3 had died) in the normal FPG group. Compared with the 237 diabetic individuals who participated in the study, the 94 who did not participate had higher glucose (but not HbA1c) levels and higher systolic blood pressure at inclusion but did not differ according to age or sex; no differences were seen between those who did and did not participate in the IFG or the normal FPG groups. At the 10-year examination, 8 of the participants selected for the IFG group were treated for diabetes, so they underwent analysis in the diabetes group.

We excluded from this analysis the 18 individuals for whom the photographs were not interpretable for either eye, and we excluded an additional 15 participants for whom a photograph for only 1 eye was interpretable; they had baseline characteristics similar to those included in the analysis, except that those excluded were older (P < .001). Ten participants were in the diabetes group, 11 in the IFG group, and 12 in the normal FPG group.

Our analysis thus included 700 participants: 235 with diabetes (145 treated for diabetes, of whom 36 were treated for diabetes at baseline and 90 had at least 1 FPG measurement of ≥126 mg/dL), 227 with IFG at some time during the study, and 238 with glucose levels within reference limits during the entire study.

The DESIR Study was approved by the Kremlin-Bicêtre Hospital ethics committee, Le Kremlin-Bicêtre, and participants signed an informed consent.

METHODS OF MEASUREMENTS AT BASELINE

At baseline, blood pressure was measured in a supine position after 5 minutes of rest and waist circumference (the smallest circumference between the lower rib and the iliac crests) was determined. Weight and height were measured in lightly clad participants, and the body mass index (BMI) was calculated.

All biochemical measurements were from 1 of 4 health center laboratories at La Riche, Blois, Chartres, or Orleans. We measured FPG level using the glucose-oxidase method with commercially available analyzers (Technicon RA100 [Bayer Diagnostics, Puteaux, France] or Specific [Konelab, Evry, France]). Levels of HbA1c were measured using an automated high-performance liquid chromatography ion-exchange analyzer (Hitachi/Merck-VWR; Bayer Diagnostics) or an automated immunoassay system (DCA 2000; Bayer Diagnostics). To adjust for differences between laboratories, glucose and HbA1c, data were standardized in age and sex strata with respect to reference data assayed in the La Riche laboratory. Serum insulin levels were quantified centrally using a particle enzyme immunoassay with an automated analyzer (Micro with IMX; Abbott, Rungis, France). Levels of total cholesterol, high-density lipoprotein cholesterol, and triglycerides were assayed by one of 2 commercial kits (DAX 24 [Bayer Diagnostics] or KONE [Konelab]); low-density lipoprotein cholesterol levels were determined by the Friedewald equation. We assessed C-reactive protein (CRP) levels by the immunonephelometric method (Dade Behring, Marburg, Germany) and were available for only 206 participants at baseline and at the 3-year follow-up, but were available for all participants at the 6- and 9-year follow-up examinations. The interlaboratory variability was assessed monthly on normal and pathological values for each biological variable, and the coefficients of variation for laboratories were less than 6% during the inclusion period.

Albuminuria was determined from a single-void sample by nephelometry (BNA Behring, Rueil-Malmaison, France) in participants without dipstick findings positive for hematuria. Microalbuminuria or macroalbuminuria was defined as albuminuria of 20 mg/L or greater or dipstick findings positive for proteinuria.

Participants completed a self-administered questionnaire on whether they had a family history of diabetes or drug treatment for diabetes, hypertension, or lipid levels. The presence of clinical retinopathy was not recorded at baseline.

RETINAL PHOTOGRAPHY

We used a nonmydriatic digital retinal camera (TRC-NW6; Topcon, Rotterdam, the Netherlands) that allows color photographs to be taken without papillary dilation and uses semiautomatic guidance for peripheral fixation. Images were captured in true color (24 bits) at a resolution of 1490 × 960 pixels. Retinal photographs were taken by a qualified orthoptist who was specifically trained for this study. The room was well-darkened, and 3 photographs were taken, including 1 centered on the macula, 1 on the optic disc, and 1 temporal to the macula. The right eye was always photographed first, after 5 minutes of adaptation to the dark. Participants were asked to close their eyes for a few seconds between each photograph. After an interval of 2 to 3 min-


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More than 70% of the participants with diabetes mellitus at 9 years were men; thus, in this study, 72% were men owing to matching by sex (Table 1). The average age at baseline was 52 years. A total of 44 participants...
were classified as having retinopathy, including 19 with diabetes (5 treated at baseline, 15 treated at the 10-year examination, and 4 with FPG levels of ≥126 mg/dL during the study), 19 with IFG levels, and 6 with normal glucose levels throughout the 9 years of the study. In most participants, retinopathy was mild and limited to microaneurysms and/or hemorrhages. Those with retinopathy were more often treated for diabetes at baseline than those without retinopathy (11.4% vs 4.7%; \( P = .06 \)) (Table 1), and they had higher baseline mean FPG and HbA\(_1c\) levels (\( P < .001 \)), with a trend for a higher systolic blood pressure (142 vs 137 mm Hg; \( P = .06 \)), and a higher percentage were treated for hypertension (36.4% vs 19.6%; \( P = .008 \)). Of the 6 participants who were always normoglycemic but who had signs of retinopathy, 5 were women; they had age, BMI, blood pressure, and lipid variables similar to the other 38 participants with retinopathy. None of these 6 had cardiovascular disease.

### Table 2. Average of Area Under the Curve per Year During the 9 Years of Follow-up for Various Characteristics Associated With the Absence or Presence of Retinopathy at 10 Years

<table>
<thead>
<tr>
<th>Group, Mean (SD)</th>
<th>No Retinopathy</th>
<th>Retinopathy</th>
<th>( P ) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BM I</td>
<td>26.8 (4.1)</td>
<td>27.8 (3.6)</td>
<td>.12</td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>92 (11)</td>
<td>94 (10)</td>
<td>.14</td>
</tr>
<tr>
<td>Glucose level, mg/dL</td>
<td>110 (21)</td>
<td>132 (40)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>HbA(_1c) level, %</td>
<td>5.9 (0.7)</td>
<td>6.5 (1.4)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Insulin level, ( \mu U/mL )</td>
<td>9.8 (6.0)</td>
<td>11.2 (7.0)</td>
<td>.14</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>140 (15)</td>
<td>143 (18)</td>
<td>.11</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>83 (8)</td>
<td>84 (9)</td>
<td>.42</td>
</tr>
<tr>
<td>Triglyceride level, mg/dL</td>
<td>125 (75)</td>
<td>147 (89)</td>
<td>.04</td>
</tr>
<tr>
<td>HDL cholesterol level, mg/dL</td>
<td>58 (13)</td>
<td>54 (13)</td>
<td>.11</td>
</tr>
<tr>
<td>LDL cholesterol level, mg/dL</td>
<td>141 (26)</td>
<td>147 (25)</td>
<td>.14</td>
</tr>
<tr>
<td>CRP level, mg/L</td>
<td>2.5 (2.6)</td>
<td>3.2 (1.9)</td>
<td>.07</td>
</tr>
</tbody>
</table>

**Abbreviations:** See Table 1.

**SI conversion factors:** See Table 1.

**a** Indicates logarithms used for statistical analyses.

**b** Two hundred six participants were included: 193 for the group without and 15 for the group with retinopathy.

During the 9-year follow-up, the area under the curve analysis showed that FPG and HbA\(_1c\) levels were the factors most associated with retinopathy at 10 years (\( P < .001 \)), and triglyceride levels were higher in those with retinopathy (147 vs 125 mg/dL; \( P = .04 \). Table 2) (to convert to millimoles per liter, multiply by 0.0113). Levels of CRP were analyzed in only 206 of the participants and showed a marginal relationship with retinopathy; the average areas under the curve for the 6- and 9-year examinations, when data were available for all participants, were 3.4 vs 2.7 mg/L (\( P = .01 \)) (to convert to nanomoles per liter, multiply by 9.524) for those with and without retinopathy.

The overall frequency of retinopathy at 10 years, standardized to the baseline glucose status in the study, was 3.6%, and there was a sharp increase in frequency in the group with the highest baseline glucose levels (FPG level of ≥126 mg/dL; Figure 1A), with a significant difference in frequency between the last 2 glucose groups (\( P = .04 \)). For the HbA\(_1c\) groups, the frequency of retinopathy also increased in a similar fashion (Figure 1B), with a statistically significant difference between the last 2 HbA\(_1c\) groups (\( P = .02 \)). When individuals with drug-treated diabetes at baseline were excluded from the analysis, the relationships changed little (Figure 1).

For the positive predictive values (Figure 2), there were essentially 2 parts to the relationships: a very gradual increase, then a higher slope after around 108 mg/dL for FPG and around 6.0% for HbA\(_1c\) levels. These could be considered as potential thresholds for defining those at risk of later retinopathy.

An FPG level of 108 mg/dL had a positive predictive value of 8.4%, a negative predictive value of 97%, sensitivity of 27%, specificity of 88%, and a positive likelihood ratio of 2.4. For an HbA\(_1c\) level of 6.0%, the corresponding values were 6.0%, 98%, 19%, 92%, and 1.8, respectively. For a glucose threshold of 116 mg/dL, these values were 14.0%, 96%, 19%, 97%, and 4.3, respectively; for an HbA\(_1c\) level of 6.5%, they were 14.8%, 97%, 9%, 98%, and 4.8, respectively. Once again, little change was observed when patients with diabetes at baseline were excluded. The ROC curves were fairly shallow for FPG.
and HbA1c levels (Figure 3), and the area under the ROC curve was 64% for both measures.

The frequencies (95% confidence intervals) of 10-year retinopathy were 2.5% (0.5%-4.5%) in participants who were always normoglycemic, higher in those with IFG levels at some time during the 10-year follow-up (8.6% [5.0%-12.2%]; \( P < .001 \)), similar to the 7.9% (4.4%-11.3%) (\( P = .01 \) when compared with participants in the normoglycemic group) in participants with diabetes at the end of follow-up (Figure 4). The frequency (95% confidence interval) of retinopathy in the 36 individuals treated for diabetes at baseline was 13.9% (2.6%-25.2%), compared with a frequency of 6.7% (3.2%-10.3%) in the 199 participants who became diabetic during the 10 years of the study.

The Pearson correlation coefficient between the logarithms of FPG and HbA1c levels was quite high (0.73). In univariate regression models, the FPG level was more closely associated with retinopathy than was the HbA1c level (\( \chi^2 \) test Wald statistics, 26.75 and 18.81, respectively; both \( P < .001 \)). There was no improvement after adding HbA1c levels to the model with FPG levels alone. Levels of FPG and HbA1c remained predictive of 10-year retinopathy (both, \( P < .001 \)) after adjusting for age, sex, hypertensive treatment, and systolic blood pressure, none of which was significant. With the area under the ROC curve used as a measure to discriminate between those with and without 10-year retinopathy, the areas were 64% for FPG level alone, as well as for HbA1c level alone or for the combination of risk factors (age, sex, hypertensive treatment, and systolic blood pressure); thus, all 3 models discriminated equally. Adding FPG level to the basic risk factors increased the area to 72%, whereas adding HbA1c the increase was lower at 69% and adding HbA1c level to the model with FPG level

![Figure 2](http://archophth.jamanetwork.com/pdfaccess.ashx?url=/data/journals/ophth/10232/ on 06/26/2017)
and the risk factors left the area at 72%. Thus, addition of HbA1c level did not improve the discrimination.

**COMMENT**

The positive predictive values for retinopathy increased very slowly for baseline FPG and HbA1c levels at lower values and then increased more rapidly at the values we might consider indicative of diabetes risk: approximately 108 mg/dL for FPG level and 6.0% for HbA1c level. It is more difficult to discern a threshold after which the frequency of retinopathy increased because the frequency can be shown only by grouping the glycemic measures into intervals. The positive predictive values provide more information about possible thresholds. Furthermore, we emphasize the use of the positive predictive values because they are more clinically relevant for finding thresholds to predict those who will have retinopathy. Our study included volunteers for clinical retinopathy, we are not able to study the incidence of retinopathy. Our study included volunteers for a free health checkup who agreed to participate in the 9-year DESIR Study and then agreed to an additional examination to study diabetic microvascular disease. This type of self-selection is a common limitation in studies in the general population but should not change the relationships between the glycemic variables or other risk factors and the outcome. Our sample size permitted us to find only those risk factors with a strong association with retinopathy.

The American Diabetes Association and the World Health Organization use an FPG level of 126 mg/dL or greater to define diabetes.1,22 This level is based on 3 pivotal studies that showed that signs of retinopathy were rare at an FPG level of less than 126 mg/dL but that the prevalence increased substantially above that level. Those studies were limited by the use of poor methods for detecting diabetic retinopathy that were based on ophthalmoscopy or single-field photography. Reevaluating this threshold in 3 contemporary studies and using multiple retinal photographs and a validated retinal grading system, Wong et al1 found that the prevalence of retinopathy increased with FPG concentration, without a clear diagnostic cutoff. They also found that signs of retinopathy occur in 7% to 13% of the population at an FPG concentration of less than 126 mg/dL. For HbA1c level, a large study from Malaysia recommended a threshold value of 6.6% to 7.0% for diagnosing diabetes.5

Most reports on prevalent and incident retinopathy study glycemic factors only. Other factors shown to be associated with prevalent retinopathy include age; hypertension; use of hypertensive medications; BMI; levels of insulin, cholesterol, triglycerides, and C-peptide; the ratio of urinary albumin to creatinine; inflammation; and endothelial dysfunction.7,10,12-23 Incident retinopathy was associated with waist-hip ratio in the Hoorn Study.24 In the DESIR Study, only hypertensive treatment at baseline was related to retinopathy at 10 years, but, for the cumulative effects during the 9 years of the study, evaluated by the area under the curve for this period, there was a significant association only with higher levels of triglycerides. Marginal associations (P < .14) were also seen with BMI, waist circumference, systolic blood pressure, and levels of insulin, high- and low-density lipoprotein cholesterol, and CRP.

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**Figure 3.** Receiver operating characteristic curves for 10-year retinopathy according to baseline fasting plasma glucose (FPG) concentration and baseline hemoglobin A1c (HbA1c) level.**

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**Figure 4.** Frequency (95% confidence interval) of retinopathy 10 years after baseline according to glucose status during the 10-year study. FPG indicates fasting plasma glucose; IFG, impaired fasting glucose.
Few longitudinal studies of retinopathy have been performed, and they all show a relationship with glucose or HbA1c levels. In the largest single study,25 of incident retinopathy, diagnosed from fundus photographs, the incidence increased from 15 cases per 10,000 person-years for FPG levels of less than 90 mg/dL to around 30 cases for glucose levels of 90 to 99, 100 to 109, and 110 to 125 mg/dL; incidence then increased rapidly for the 3 glucose classes within the diabetic range. In the DESIR Study, we were able to show a significant difference in retinopathy frequency at 10 years, between baseline FPG levels of less than 126 mg/dL and those of 126 mg/dL or greater. In the Pima Indian Study,26,27 5-year incident retinopathy increased at FPG and HbA1c values of about 126 mg/dL and 7.0%, respectively. Incident retinopathy during 9.4 years in the Hoorn Study28 was more than 2-fold higher in the diabetic patients than in the normoglycemic population. The incidence of retinopathy was more than 3-fold higher in the upper third of the population with HbA1c levels of 5.8% or greater than in the remainder of the population. In the DESIR Study, the 10-year prevalence of retinopathy was 3.3% for HbA1c levels of less than 6.0% and 6.8% for those with an HbA1c level of 6.0% or greater. In the Atherosclerosis Risk in Communities Study,29 the 3-year incidence of any retinopathy was 3 times higher for individuals in the upper quartile of the FPG distribution at baseline (≥114 mg/dL) compared with those in the lowest quartile group (<95 mg/dL). Another publication from Australia30 showed that the 5-year incidence of any retinopathy increased at approximately 142 mg/dL for FPG level. It is difficult to compare actual glucose and HbA1c levels at which the frequency of retinopathy increases in these different studies because there probably were differences in the accuracy of these assays for glucose and especially for HbA1c levels. The standardization of the HbA1c assay results should improve this situation for future studies.27

Levels of HbA1c and FPG at baseline were related to the presence of retinopathy 10 years later, and the levels at which the positive predictive values increased provide a rationale for the choice of thresholds for the definition of hyperglycemia associated with 10-year retinopathy. We propose that thresholds of 108 mg/dL for FPG concentration and 6.0% for HbA1c level could be used to define those who are at risk of retinopathy; this is in agreement with our observation of a risk of retinopathy within the IFG range (FPG level, ≥110 mg/dL). Factors other than glucose measures play only a minor role in retinopathy.

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Disclaimer: The sponsors were not involved in the analysis or interpretation of the data.

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REFERENCES


Ophthalmological Numismatics

Following the completion of his medical degree in 1890 at Brussels University, Emile Gallemaerts (1860-1935) did clinical work until succeeding his mentor Jean-Baptiste Coppez as the head of the Brussels University Department of Ophthalmology in 1905, a post he held until his forced retirement at 65 years of age in 1925. The year of his retirement, Gallemaerts was also president of the Belgian Academy of Medicine. His publications focused both on histopathological problems and clinical work. Among his more valuable contributions were the detection of magnetic intraocular bodies with a magnetometer and the use of the ocular slitlamp biomicroscope. He is considered the founder of the Belgian Ophthalmological Society.

In 1925, a lifetime portrait medal by Jules Lagaë measuring 60 mm in diameter was struck in bronze in honor of Gallemaerts’ retirement, from his students and friends. The obverse depicts his bust facing right. The reverse has an honorific inscription in 8 lines.

Courtesy of: Jay M. Galst, MD, Clinical Associate Professor, New York Medical College, and Peter van Allen, PhD, Associate Curator, American Numismatic Society.

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