Hemoglobin A1c Level and Future Cardiovascular Events Among Women

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Background: Available data suggest that hemoglobin A1c (A1c), also known as glycosylated hemoglobin, levels may be related to cardiovascular risk in the general population without diabetes mellitus. We sought to test this hypothesis prospectively in a cohort of women without overt cardiovascular disease.

Methods: We conducted a nested case-control study of the Women’s Health Study cohort. We identified 464 case patients with incident myocardial infarction, stroke, or coronary revascularization and 928 unmatched control subjects who remained free of cardiovascular events at case diagnosis. The mean follow-up was 7 years.

Results: Of the overall study population, 136 had a history of diabetes mellitus or an overtly elevated baseline A1c level (≥6.4%) and were excluded from the primary analyses. Among women without diabetes mellitus or an elevated baseline A1c level, mean±SD baseline levels of A1c were significantly higher among future cases than controls (5.47%±0.27% vs 5.37%±0.22%; P=.001). The crude relative risks (RRs) of incident cardiovascular events for increasing quartiles of A1c were 1.00, 0.98, 1.33, and 2.25 (95% confidence interval [CI] for the highest vs the lowest quartile, 1.59-3.18). The A1c levels correlated with several other traditional cardiovascular risk factors, and in fully adjusted models, the predictive effect of A1c was attenuated and not significant (RR for the highest vs the lowest quartile, 1.00; 95% CI, 0.65-1.54). In contrast, in the population including women with diabetes mellitus at enrollment, diabetes mellitus (RR, 4.97; 95% CI, 2.81-8.77) remained a strong independent determinant of cardiovascular risk in fully adjusted analyses, while A1c levels did not (RR for the highest vs the lowest quartile, 1.11; 95% CI, 0.73-1.71).

Conclusions: The A1c level is associated with future cardiovascular risk among women without diabetes mellitus, but this relationship is largely attributable to a strong correlation with other cardiovascular risk factors. In contrast, diabetes mellitus is a strong independent determinant of cardiovascular risk, even after adjustment for A1c levels.

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Patients with diabetes mellitus are known to be at markedly increased risk for cardiovascular disease.1,2 While the precise mechanism through which diabetes mellitus confers increased cardiovascular risk remains unclear, poor glycemic control is thought likely to contribute.3,6 Furthermore, available data suggest that even modest elevations in blood glucose, much lower than those required to diagnose diabetes mellitus, may be associated with increased cardiovascular risk.7-10

The hemoglobin A1c (A1c), also known as glycosylated hemoglobin, level is an indicator of average blood glucose concentration during the prior 2 to 3 months. Data regarding A1c levels and future cardiovascular risk among individuals without diabetes mellitus are sparse, especially among women, but available data suggest that A1c levels may predict incident cardiovascular events, even among patients without diabetes mellitus.11-13

We sought to determine prospectively if baseline levels of A1c were a predictor of incident cardiovascular events among a large population of women without diabetes mellitus who were free from overt cardiovascular disease, and to compare the predictive value of A1c level and diabetes mellitus for incident cardiovascular events, after the inclusion of women with diabetes mellitus at baseline.

Methods: The Women’s Health Study is an ongoing, randomized, double-blind, placebo-controlled trial of aspirin and vitamin E being conducted among middle-aged female health profession-
als with no history of cardiovascular disease or cancer. At baseline, blood samples were collected in tubes containing EDTA from 28,349 women, and stored in liquid nitrogen until analysis. Questionnaires were sent to Women’s Health Study participants to elicit information on cardiovascular risk factors and incident cardiovascular events. For this analysis, case subjects were study participants from whom a baseline blood sample was obtained and who subsequently had a cardiovascular event before confirmed myocardial infarction, stroke, or coronary revascularization (percutaneous coronary intervention or coronary artery bypass surgery). The mean follow-up was 7 years.

For all cases of myocardial infarction, stroke, or coronary revascularization, hospital records were obtained and reviewed. Myocardial infarction was classified as confirmed if symptoms met the criteria of the World Health Organization and if the event was associated with abnormal levels of cardiac enzymes or diagnostic electrocardiographic changes. Reported stroke was confirmed if the patient had a new neurological event persisting for more than 24 hours or until death; computed tomographic scans or magnetic resonance images were available for most women who experienced a stroke.

For each woman with a confirmed cardiovascular event during follow-up, 2 control subjects were selected from among the remaining study participants from whom a baseline blood sample had been obtained and who remained free of reported cardiovascular events when the case was diagnosed. The controls were not matched on any risk factors. With the use of these criteria, 464 cases and 928 controls were selected. The cases comprised 136 women who experienced a myocardial infarction (8 of which were fatal), 165 women who experienced a stroke (13 of which were fatal), and 163 women who underwent coronary revascularization.

ASSAYS

Baseline plasma samples were thawed and assayed for total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), triglycerides, and direct low-density lipoprotein cholesterol levels on an analyzer (Hitachi 911, Roche Diagnostics, Indianapolis, Ind) with reagents (Roche Diagnostics; and Genzyme Corp., Cambridge, Mass). Plasma levels of C-reactive protein (CRP) were also measured using a validated high-sensitivity assay (Denka Seiken, Niigata, Japan).

The A1c levels were determined on an analyzer (Hitachi 911) based on turbidimetric immuno-inhibition using packed red blood cells (Roche Diagnostics). The A1c antibodies in the reagent react specifically with A1c in the sample and form soluble antigen-antibody complexes. Polyethylene glycols are then added to bind excess antibodies, and the resulting agglutinated complex is measured turbidimetrically. The amount of A1c in the sample is inversely proportional to the amount of turbidity formed. This assay is approved by the US National Glycohemoglobin Standardization Program and by the Food and Drug Administration for clinical use. The day-to-day variabilities at A1c values of 5.3 and 9.1 g/dl are 1.9% and 3.0%, respectively. One case and one control were missing A1c values because of inadequate samples.

For all biochemical and A1c analyses, samples were handled in a fully blinded fashion such that all investigators had no knowledge of case or control status.

STATISTICAL ANALYSIS

Given the high prevalence of undiagnosed diabetes mellitus in the general population, we performed our primary analyses after the exclusion of women diagnosed as having diabetes mellitus or an elevated A1c level (≥6.4%) at baseline. To compare the predictive value of diabetes mellitus and A1c level, we also repeated analyses for the total study population, including women with diabetes mellitus or an elevated A1c level at baseline. Means and proportions for risk factors for cardiovascular events at baseline were calculated for cases and controls. The t test was used to evaluate differences in means, and the χ² statistic was used to compare proportions. For CRP level, medians were compared using the Wilcoxon rank sum test. An analysis of trends was used to test for any association between increasing levels of each plasma marker and the risk of future cardiovascular events, after the sample was divided into quartiles according to the distribution of A1c among the control subjects. All models were adjusted for random assignment to aspirin or vitamin E. The Spearman rank correlation coefficient was used to assess the correlation between A1c level and other risk factors among control subjects.

Adjusted risk estimates were obtained with the use of logistic regression models that adjusted for random assignment to aspirin or vitamin E in the Women’s Health Study and several other risk factors for cardiovascular events, including age, current smoking status, TC/HDL-C ratio, systolic blood pressure, body mass index, CRP level, a parental history of myocardial infarction before the age of 60 years, use of hormone therapy, and a history of diabetes mellitus at enrollment (for the total population only). We used the C statistic to estimate the area under the receiver operating characteristic curve and the Hosmer-Lemeshow goodness-of-fit test to assess the goodness of fit for this fully adjusted model. The data set was complete for 1271 cases and controls in this final adjusted model. All P values were 2-tailed, and P <.05 was considered statistically significant. All confidence intervals were calculated at the 95% level.

RESULTS

The baseline clinical characteristics of the women who subsequently developed cardiovascular events and those who remained free of cardiovascular events are shown in Table 1. As expected, cases were older, were more likely to be current smokers, and tended to be more likely to have a family history of premature myocardial infarction. Baseline levels of systolic and diastolic blood pressure, body mass index, TC/HDL-C ratio, triglycerides, and CRP were also significantly higher among cases than controls. The current use of hormone therapy did not differ between cases and controls.

One hundred thirty-six women (40 controls and 96 cases) had either diabetes mellitus or an A1c level greater than 6.4% at baseline. After exclusion of these women (Table 1), the mean ± SD baseline levels of A1c were higher among future cases than controls. In the total study population including those women with diabetes mellitus or an elevated A1c level at baseline (Table 1), the mean ± SD baseline levels of A1c were also significantly higher among cases than controls, and diabetes mellitus at enrollment was more prevalent among future cases than controls. The crude odds ratio for incident cardiovascular events associated with diabetes mellitus was 6.97 (95% confidence interval, 4.40-11.05) (P < .001).

The A1c levels were significantly correlated with several other traditional cardiovascular risk factors, including age, body mass index, systolic blood pressure, CRP level, and TC/HDL-C ratio (Table 2). The impact of controlling for the confounding effects of these and other risk factors is shown in Table 3. Among women without dia-
In contrast, in this fully adjusted model including A1c level, (RR for the highest vs the lowest quartile, 1.22; P=.30), the effect of baseline levels of A1c was substantially attenuated and no longer significant after adjustment for age and smoking status (RR for the highest vs the lowest quartile). This association between A1c levels and cardiovascular events among women without diabetes mellitus was substantially attenuated and no longer significant after adjustment for age and smoking status (RR for the highest vs the lowest quartile, 1.00; P=.99).

For the overall study population (Table 3), the crude relative risks (RRs) of incident cardiovascular events for increasing quartiles of A1c were 1.00, 0.98, 1.33, and 2.25, respectively (P<.001 for the highest vs the lowest quartile). This association between A1c levels and cardiovascular events among women without diabetes mellitus was substantially attenuated and no longer significant after adjustment for age and smoking status (RR for the highest vs the lowest quartile, 1.22; P=.30). There was no evidence of association after adjustment for age, smoking status, TC/HDL-C ratio, body mass index, current use of hormone therapy, family history of premature myocardial infarction, and CRP level (RR for the highest vs the lowest quartile, 1.00; P=.99).

For the overall study population (Table 3), the crude RRs of incident cardiovascular events for increasing quartiles of A1c were 1.00, 0.96, 1.28, and 3.31, respectively (P<.001 for the highest vs the lowest quartile). Adjustment for age and smoking status somewhat attenuated the magnitude of the RR for the highest compared with the lowest quartile (RR, 2.08; P<.001). However, in a fully adjusted model including diabetes mellitus, the RRs for increasing quartiles of A1c were 1.00, 0.75, 0.93, and 1.11, respectively (P=.60 for the highest vs the lowest quartile). In contrast, in this fully adjusted model including A1c level, a diagnosis of diabetes mellitus at enrollment remained a strong predictor of incident cardiovascular events (RR, 4.97; 95% confidence interval, 2.81-8.77; P<.001). The area under the receiver operating characteristic curve for this fully adjusted model was 0.81, and the Hosmer-Lemeshow goodness-of-fit test for the model failed to reject the null hypothesis (P=.90).

Finally, in analyses restricted to those 136 women with either diabetes mellitus or elevated A1c levels at baseline, the mean±SD baseline levels of A1c did not differ significantly among cases (n=96) vs controls (n=40) (7.99%±1.65% vs 7.90%±1.89%; P=.78).

We sought to determine if baseline levels of A1c were associated with future cardiovascular risk among a large population of women without diabetes mellitus. We found that baseline levels of A1c were a strong predictor of future cardiovascular risk among a large population of women without diabetes mellitus. We found that baseline levels of A1c were a strong predictor of future cardiovascular risk among a large population of women without diabetes mellitus.
Table 3. Crude and Adjusted Relative Risks of Future Cardiovascular Events According to Baseline Quartile of A1c Level*

<table>
<thead>
<tr>
<th>Quartile of A1c Level (% Range)</th>
<th>Relative Risk (95% Confidence Interval)†</th>
<th>Fully Adjusted Model‡</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Women Without Diabetes Mellitus or an A1c Level &gt;6.4% at Baseline</td>
<td>Total Study Population</td>
</tr>
<tr>
<td></td>
<td>Crude</td>
<td>Adjusted for Age and Smoking</td>
</tr>
<tr>
<td>1 (&lt;5.23)</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>2 (5.23-5.35)</td>
<td>0.98</td>
<td>(0.66-1.45)</td>
</tr>
<tr>
<td>3 (5.36-5.49)</td>
<td>1.33</td>
<td>(0.92-1.93)</td>
</tr>
<tr>
<td>4 (=5.50)</td>
<td>2.25</td>
<td>(1.59-3.18)</td>
</tr>
<tr>
<td>P value for trend</td>
<td>&lt;.001</td>
<td>.006</td>
</tr>
</tbody>
</table>

*Data for A1c level were missing for 1 case and 1 control. A1c is explained in the first footnotes to Tables 1 and 2.
†All models were adjusted for random assignment to aspirin or vitamin E.
‡This model also controls for the effects of age, smoking status, body mass index, total cholesterol–high-density lipoprotein cholesterol ratio, C-reactive protein level, systolic blood pressure, family history of premature myocardial infarction, current use of hormone therapy, and diabetes mellitus (in the total study population).

As expected, a diagnosis of diabetes mellitus at enrollment into the study was a strong predictor of incidence cardiovascular events in crude analyses. However, levels of A1c were correlated with many traditional cardiovascular risk factors, and after adjustment for the confounding effects of these risk factors, baseline levels of A1c were no longer predictive of cardiovascular risk. In contrast, the presence of diabetes mellitus at enrollment remained a strong independent predictor in the overall study population, even after adjustment for A1c levels.

Prior mortality studies among populations mainly without diabetes mellitus had suggested that the predictive value of A1c level persisted in adjusted analyses. Potential differences in study design that may partly account for these disparities include sex differences, the many cases in the present study, the use of different A1c assays, and the use of different cardiovascular end points (myocardial infarction, stroke, and revascularization vs cardiovascular-related mortality). In this regard, our results were similar when our analyses were restricted to the separate cardiovascular end points (data not shown).

The finding that the highest quartile of A1c level remained a significant predictor of risk after adjustment for age and smoking status in the overall population is of interest. The presence of an elevated A1c level, through its association with the metabolic syndrome, may potentially predate the development of other risk factors, such as dyslipidemia and hypertension, and hence these risk factors may represent a common biological proatherogenic pathway. Thus, including all these risk factors together in the fully adjusted model may potentially be “overcontrolling” for the confounding effect of these variables on the predictive value of A1c level.

As expected, a diagnosis of diabetes mellitus at enrollment into the study was a strong predictor of incident cardiovascular events in the overall study population. Indeed, in the fully adjusted model, diabetes mellitus remained a strong independent predictor, even after adjustment for A1c levels. Furthermore, in analyses confined to the 136 women with diabetes mellitus or an overtly elevated A1c level at baseline, baseline levels of A1c were similar among future cases and controls. Although the latter observation is underpowered to base firm conclusions on, these data suggest that other proatherogenic effects of diabetes mellitus may be more important in determining macrovascular risk than glycemic control per se, a contention supported by the results of the large UK Prospective Diabetes Study. Further studies are required to specifically address this issue.

Our study has several limitations. First, the study cohort consists of middle-aged women without overt cardiovascular disease at baseline, and these results should not be generalized to other populations. Second, because of our study design, we used a single A1c value for our analyses and we do not have data regarding fasting glucose or 2-hour postprandial glucose levels. Third, given the high prevalence of undiagnosed diabetes mellitus, it is likely that some of our study population had undiagnosed diabetes mellitus at enrollment. Nevertheless, to minimize this effect, we excluded women with A1c levels greater than 6.4% at baseline for our primary analyses. Finally, use of frozen samples may have theoretically affected our results. However, any random misclassification of the exposure would bias our results toward the null hypothesis, and baseline A1c levels were a strong predictor of cardiovascular risk in univariate analyses.

In conclusion, in crude analyses, baseline levels of A1c were a strong predictor of cardiovascular risk in a large cohort of generally healthy women without diabetes mellitus. However, the predictive value of A1c level was largely attributable to its association with other risk factors, such that in fully adjusted models the predictive value of A1c level was fully attenuated and not significant. In contrast, diabetes mellitus at enrollment remained a strong independent predictor of risk in the overall population, even after adjustment for A1c levels. These data support the need for further research to investigate the temporal relationship between glycemic control and the development of other cardiovascular risk factors, and suggest that...
other proatherogenic effects of diabetes mellitus, rather than levels of glycemia, may be more directly related to future cardiovascular risk.

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