Glycated Hemoglobin Level Is Strongly Related to the Prevalence of Carotid Artery Plaques With High Echogenicity in Nondiabetic Individuals

The Tromsø Study

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Background—High levels of HbA1c have been associated with increased mortality and an increased risk of atherosclerosis assessed as carotid intima-media thickness or plaque prevalence. In the present population-based study, we examined the association between HbA1c and plaque prevalence with emphasis on plaque echogenicity in subjects not diagnosed with diabetes.

Methods and Results—HbA1c measurements and ultrasonography of the carotid artery were performed in 5960 subjects (3026 women, 2934 men) 25 to 84 years of age. Plaque morphology was categorized into 4 groups from low echogenicity (soft plaque) to strong echogenicity (hard plaque). HbA1c was categorized into 5 groups: <5.0%, 5.0% to 5.4%, 5.5% to 5.9%, 6.0% to 6.4% and >6.4%. Carotid plaque prevalence increased with increasing HbA1c level (P for linear trend=0.002). The OR for hard plaques versus no plaques was 5.8 in the highest HbA1c group (>6.4%) compared with subjects in the lowest group (<5.0%) after adjustment for several possible confounders. The risk of predominantly hard plaques was also significantly associated with HbA1c levels, although the ORs at each level were somewhat lower than for hard plaques. With respect to the risk of soft plaques versus no plaques, no statistically significant relationship with HbA1c levels was found.

Conclusions—Metabolic changes reflected by HbA1c levels may contribute to the development of hard carotid artery plaques, even at modestly elevated levels. (Circulation. 2004;110:466-470.)

Key Words: carotid arteries ■ plaque ■ hemoglobin A, glycosylated ■ ultrasonics

Individuals with clinically recognized diabetes mellitus are at increased risk of coronary, cerebrovascular, and peripheral artery disease. It has also been shown that the intima-media thickness (IMT) of the carotid artery, an indicator of general atherosclerosis, is increased in patients with diabetes mellitus compared with nondiabetic subjects.1 Furthermore, the progression of atherosclerotic plaques (a measure of more advanced atherosclerosis) is increased in subjects with type 2 diabetes mellitus.2

HbA1c is an indicator of average glycemia over the previous 6 to 8 weeks, and the level of HbA1c has been suggested as a diagnostic or screening tool for diabetes.3,4 Previous studies have shown that HbA1c concentration is related to mortality and cardiovascular disease in nondiabetic persons.5,6 Few studies have examined the possible relationship between HbA1c and atherosclerosis in subjects not diagnosed with diabetes.9-14 In these studies, high levels of HbA1c were associated with raised atherosclerotic lesions and more extensive fatty streaks in the coronary artery,12 and increasing levels were positively associated with carotid IMT10,13,14 and carotid plaque prevalence.11 In 1 study on normoglycemic subjects, HbA1c levels were not related to the development of plaques.9

With B-mode ultrasound, not only IMT and the prevalence of plaque but also some of the morphological intraplaque structures can be evaluated. Plaques with a high content of lipid, necrotic debris, and/or hemorrhage (echolucent, "soft" plaques) reflect ultrasound poorly, whereas plaques with a higher content of dense fibrous tissue and calcified material (echogenic, "hard" plaques) reflect ultrasound strongly. Recent population-based studies have indicated that soft stenotic plaques harbor a higher risk of clinical cerebrovascular disease than hard stenotic plaques.15,16 Whether these morphological structures could be related to metabolic changes reflected by HbA1c levels has not been examined previously.
Thus, in the present population-based study, we examined the prevalence of carotid artery plaques, with emphasis on plaque morphology, in subjects at different HbA1c levels. Only subjects without known diabetes were included.

Methods

The Tromsø Study is a population-based study of inhabitants in the municipality of Tromsø. In the fourth survey in 1994 to 1995, all inhabitants ≥25 years of age were invited to a screening, and 27 159 subjects (77%) participated. The study was approved by the regional ethics committee, and all subjects have given informed consent. Measurements of height, body weight, blood pressure, and nonfasting serum lipids were done, and information about smoking habits, prevalent diabetes mellitus, angina pectoris, previous myocardial infarction, stroke, treatment for hypertension, and physical activity was collected from self-administered questionnaires.

All attending subjects 55 to 74 years of age and smaller samples of subjects 25 to 54 and 75 to 84 years of age were invited to a second visit 4 to 12 weeks later. These subjects had been identified before the first screening was started. More extended examinations, including measurements of waist and hip circumference, nonfasting serum glucose, and serum creatinine, were carried out. The Cobas Mira instrument was used to quantify HbA1c with an immunoturbidimetric method (Unimate 5 HbA1c, Hoffmann-La Roche). The normal reference range is 4.0% to 6.5%. Nonfasting plasma insulin was measured by radioimmunoassay, and in a subgroup of 3572 men and women, measurements of waist and hip circumference, nonfasting blood glucose, and serum lipids were done, and information about smoking habits, ingest of serum glucose, and physical activity were obtained from self-administered questionnaires.

Carotid atherosclerosis was assessed by use of high-resolution B-mode ultrasonography performed with an ultrasound scanner (Acuson Xp10 128 ART, upgraded) equipped with a linear-array transducer. A plaque was defined as a localized protrusion of the internal part of the vessel wall into the lumen. Maximum plaque thickness was measured online on frozen B-mode images marked with electronic calipers with measurement readout in 10ths of a millimeter.

Plaque morphology, in terms of ultrasound echogenicity was graded as follows: 1 = echoluent (soft), 2 = predominantly echolucent, 3 = predominately echogenic, or 4 = echogenic (hard) plaques. The vessel lumen was used as the reference structure for defining echolucency, and the bright echo zone produced by the media-adventitia interface in the far wall was used as reference structure for defining echogenicity. Some plaques (3%) were defined as unclassifiable because of unsatisfactory imaging quality.

The between- and within-sonographer agreement on plaque occurrence was satisfactory, with κ values of 0.72 (95% CI, 0.60 to 0.84) and 0.76 (95% CI, 0.63 to 0.89), respectively. Agreement on classification of plaque echogenicity was also substantial, with κ values of 0.73 (95% CI, 0.59 to 0.87) and 0.69 (95% CI, 0.53 to 0.83) between and within sonographers, respectively.

A total of 6727 subjects (77% of the eligible subjects) were examined by ultrasound of the carotid arteries, and in 6164 participants, both HbA1c measurements and ultrasonography of the carotid artery were performed. In the present study, we excluded persons reporting that they had diabetes and/or used medication for diabetes (n = 202) and 2 persons with very high readings of HbA1c but normal nonfasting blood glucose. Thus, 5960 subjects (3026 women, 2934 men) were included in our analyses.

Statistical Analysis

Differences between groups were tested with ANCOVA. Multiple linear regression analysis was performed to evaluate the impact of risk factors on plaque size. The subjects were divided into groups according to different HbA1c levels. We estimated the ORs for the presence of plaque versus no plaque, echogenic plaque versus no plaque, predominantly echogenic plaque versus no plaque, predominantly echoluent plaque versus no plaque, and echoluent plaque versus no plaque at the HbA1c levels using logistic regression analysis with adjustments for potential confounders. This analysis corresponds to a categorical polytomous logistic regression on the whole data set.

Among subjects with plaques, the independent relationship between plaque echogenicity and risk factors was tested by logistic regression analysis (cumulative ordinal logit model) in which plaque echogenicity (graded 1 through 4) was treated as the dependent variable and risk factors were treated as independent variables. The model refers to the OR for being in a lower (i.e., more echoluent) category of the dependent variable relative to the higher categories.

A score test confirmed that the proportional odds assumption was met (P = 0.21).

The data were analyzed with the Windows 10.0 version of SPSS and version 8 of the SAS software package.

Results

A total of 2954 participants had carotid artery plaques. One hundred eighty subjects had echogenic plaques, 1574 had predominantly echogenic plaques, 949 had predominantly echoluent plaques, and 128 had echoluent plaques. Plaques were not classified in 123 subjects.

Selected characteristics of the study group according to different HbA1c levels are presented in Table 1. Subjects with higher HbA1c levels tended to have higher body mass index, a higher waist-to-hip ratio, higher systolic blood pressure, and higher levels of serum total cholesterol but lower levels of serum HDL cholesterol. Moreover, the nonfasting serum glucose level increased by increasing HbA1c, and the duration of smoking was longer. Furthermore, the prevalence of angina pectoris was higher, and more subjects used or had used medication for hypertension at higher HbA1c levels.

Table 2 shows that the carotid plaque prevalence increased with increasing HbA1c (P = 0.002). When the echogenicity of the lesion is considered (as shown in Table 3), the OR for hard plaques relative to no plaques increased gradually, reaching 5.8 in the highest HbA1c group (>6.4%) compared with subjects in the lowest group (<5.0%) after adjustment for several possible confounders. The risk of predominantly hard plaques relative to no plaques was also significantly associated with HbA1c levels, but the association was weaker. With respect to the risk of soft plaques, there was a tendency toward an inverse relationship with HbA1c levels, although this trend was not statistically significant (P = 0.10). Note that adjustments for possible confounders in addition to age and sex did not have a major impact on the relationships.

The relationships between HbA1c level and the prevalence of hard and predominantly hard plaques were also statistically significant when the analysis was restricted to subjects with HbA1c < 6.0% (P for linear trend = 0.03 and 0.02, respectively).

In separate regression models (with multivariate adjustments), we observed that for every unit (1%) increase in HbA1c, the odds of having hard plaques and predominantly hard plaques versus no plaques were 1.62 (95% CI, 1.14 to 2.29) and 1.30 (95% CI, 1.10 to 1.53), respectively. There were no significant relationships to the risk of predominantly soft plaques and soft plaques (OR, 1.00; 95% CI, 0.82 to 1.22; and OR, 0.70; 95% CI, 0.43 to 1.14, respectively).

Moreover, in a separate cumulative ordinal logistic regression model (with multivariate adjustments) including only subjects with plaque, we observed that for each 1% increase in HbA1c, the
TABLE 1. Characteristics of Individuals Without Previously Diagnosed Diabetes in Relation to Different Levels of HbA$_{1c}$

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>&lt;5.0</th>
<th>5.0–5.4</th>
<th>5.5–5.9</th>
<th>6.0–6.4</th>
<th>&gt;6.4</th>
<th>P for Trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>55.8 (0.4)</td>
<td>59.1 (0.2)</td>
<td>62.0 (0.2)</td>
<td>63.7 (0.5)</td>
<td>64.8 (1.2)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Male gender, %</td>
<td>58</td>
<td>48</td>
<td>48</td>
<td>49</td>
<td>57</td>
<td>0.02</td>
</tr>
<tr>
<td>Body mass index, kg/m$^2$</td>
<td>25.6 (0.2)</td>
<td>25.6 (0.1)</td>
<td>26.2 (0.1)</td>
<td>27.2 (0.2)</td>
<td>29.1 (0.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td>0.86 (0.002)</td>
<td>0.87 (0.001)</td>
<td>0.87 (0.001)</td>
<td>0.89 (0.003)</td>
<td>0.92 (0.008)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>140.6 (0.8)</td>
<td>139.6 (0.4)</td>
<td>139.4 (0.4)</td>
<td>145.4 (1.1)</td>
<td>146.2 (2.4)</td>
<td>0.006</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>80.3 (0.5)</td>
<td>80.1 (0.2)</td>
<td>80.0 (0.3)</td>
<td>82.5 (0.6)</td>
<td>80.9 (1.4)</td>
<td>0.13</td>
</tr>
<tr>
<td>Serum total cholesterol, mmol/L</td>
<td>6.61 (0.05)</td>
<td>6.66 (0.02)</td>
<td>6.89 (0.03)</td>
<td>6.95 (0.07)</td>
<td>6.92 (0.15)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Serum HDL cholesterol, mmol/L</td>
<td>1.58 (0.02)</td>
<td>1.57 (0.01)</td>
<td>1.52 (0.01)</td>
<td>1.45 (0.02)</td>
<td>1.29 (0.05)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Serum creatinine levels, µmol/L</td>
<td>78.98 (0.66)</td>
<td>78.57 (0.32)</td>
<td>78.68 (0.35)</td>
<td>80.29 (0.88)</td>
<td>79.47 (1.97)</td>
<td>0.39</td>
</tr>
<tr>
<td>Non-fasting serum glucose, mmol/L</td>
<td>4.61 (0.03)</td>
<td>4.66 (0.01)</td>
<td>4.79 (0.02)</td>
<td>5.15 (0.04)</td>
<td>6.75 (0.08)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values are mean (SE) when appropriate, adjusted for age and sex.

In all our analyses, the results for men and women were merged, and adjustments for gender were undertaken. There was a tendency toward a stronger relationship between HbA$_{1c}$ levels and plaques with high echogenicity in women than in men. However, the results for women and men did not differ statistically ($P$=0.4 for any plaque or plaque with different morphology). There were also no significant interactions with age ($P$=0.2 for all plaque types).

In the logistic regression analysis (see Table 3 for results), we estimated the OR for the presence of plaques of a particular category of echogenicity versus no plaque at the different HbA$_{1c}$ levels. When we compared each plaque category with the total population (eg, the presence of echogenic plaque versus plaques with another echogenicity or no plaque at all), the picture was similar, although in this case, the inverse association between echolucent plaque and HbA$_{1c}$ level was statistically significant ($P$ for trend=0.03), and the positive association between echogenic plaque and HbA$_{1c}$ level was somewhat weaker ($P$ for trend=0.03).

In subjects with hard plaques, plaque size was associated with HbA$_{1c}$ level. In these subjects, the mean maximal thickness of hard plaque increased linearly over the HbA$_{1c}$ levels, with a mean thickness of 1.84 mm at the lowest level, increasing to means of 1.89, 2.08, 2.32, and 2.38 mm at the higher levels, respectively ($P$ for trend=0.01 after multivariate adjustments). For other plaque types, no significant associations between HbA$_{1c}$ level and plaque thickness were found ($P$ for trend $\geq$0.4).

**Discussion**

In this study, we found that the level of HbA$_{1c}$ was significantly related to the risk of carotid plaques and that the risk
TABLE 3. OR for Echogenic, Predominantly Echogenic, Predominantly Echolucent, and Echolucent Plaque Versus No Plaque According to HbA1c Levels

<table>
<thead>
<tr>
<th>HbA1c Levels, %</th>
<th>Total Without Plaque n</th>
<th>Echogenic n</th>
<th>Predominantly Echogenic n</th>
<th>Predominantly Echolucent n</th>
<th>Echolucent n</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR† (95% CI)</td>
<td>OR† (95% CI)</td>
<td>OR† (95% CI)</td>
<td>OR† (95% CI)</td>
<td></td>
</tr>
<tr>
<td>&lt;5.0</td>
<td>393</td>
<td>6</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>5.0–5.4</td>
<td>1454</td>
<td>75</td>
<td>2.66 2.68 (1.14–6.32)</td>
<td>634</td>
<td>1.14 1.15 (0.89–1.48)</td>
</tr>
<tr>
<td>5.5–5.9</td>
<td>1011</td>
<td>81</td>
<td>3.93 2.96 (1.26–6.99)</td>
<td>651</td>
<td>1.42 1.32 (1.02–1.70)</td>
</tr>
<tr>
<td>6.0–6.4</td>
<td>127</td>
<td>14</td>
<td>4.41 3.84 (1.40–10.52)</td>
<td>132</td>
<td>2.04 1.70 (1.19–2.43)</td>
</tr>
<tr>
<td>&gt;6.4</td>
<td>21</td>
<td>4</td>
<td>7.65 5.81 (1.37–24.73)</td>
<td>33</td>
<td>2.80 2.29 (1.19–4.44)</td>
</tr>
</tbody>
</table>

P for trend <0.001 0.007 <0.001 <0.001 0.23 0.77 0.17 0.10

*OR adjusted for age and sex.
†OR adjusted for age, sex, body mass index, systolic blood pressure, medication for hypertension (current and previous), total serum cholesterol, HDL cholesterol, duration of smoking, physical inactivity, and cardiovascular disease (previous myocardial infarction, angina pectoris, or stroke).

Increased continuously across increasing HbA1c levels. The relationship depended on plaque morphology in that HbA1c was positively related to the risk of hard and predominantly hard plaques but not to the risk of soft plaques. To the best of our knowledge, this is the first article to report on the relationship between HbA1c concentrations and plaque echogenicity.

Previous studies have examined the relationship between HbA1c and atherosclerosis assessed as carotid IMT thickening or the prevalence of plaques in nondiabetic subjects. Our results are in accordance with the results from most of these studies and extend them with regard to the relationship with plaque morphology. It has been suggested that the metabolic syndrome, a clustering of metabolic disorders (glucose intolerance, microalbuminuria, obesity, hypertension and lipid abnormalities), may explain the association between impaired glucose tolerance in nondiabetic subjects and atherosclerosis. In our study, however, there was an independent and strong relationship between the degree of glycemia, as reflected as HbA1c levels, and the risk of hard plaques, even after adjustment for obesity, hypertension, blood lipids, smoking, and leisure physical activity. Furthermore, although most of the subjects with HbA1c >6.4% and many of the subjects with HbA1c between 6.0% and 6.4% probably had diabetes, a possible effect of antidiabetic therapy could not have influenced our results because the disease had not been diagnosed before the study. We also note that the relationship between HbA1c and the prevalence of echogenic plaques was consistent in subjects with HbA1c <6.0%.

Because of the cross-sectional study design, we cannot formally conclude that a high HbA1c level is causally related to atherosclerosis. It has been hypothesized that type 2 diabetes mellitus may be a metabolic vascular disease with hyperglycemia as a late manifestation. On the other hand, prospective studies have shown that increasing HbA1c levels increase the risk of cardiovascular diseases among nondiabetic people. It is therefore not unlikely that the degree of glycemia also contributes to atherogenesis and that this process takes place already at modestly elevated levels of HbA1c. Furthermore, several plausible mechanisms such as oxidative stress and protein glycation of the vessel walls may link glycemic status to atherosclerosis. Moreover, it has been shown that acute hyperglycemia impairs endothelial function even in healthy people.

It is difficult to explain why HbA1c was strongly and positively related to the prevalence of hard and predominantly hard plaques but not, or if anything negatively, associated with the risk of soft plaques. It is uncertain whether soft lipid-rich plaques develop into calcified fibrotic plaques over time, but if they do, a high prevalence of hard plaques may reflect the fact that subjects with high HbA1c levels have had plaques for a longer period than euglycemic persons at the same age. Another possibility is that the progression from soft to hard plaque is accelerated in hyperglycemic subjects. Moreover, mechanisms involving fibrotic changes in the tissue (eg, activation of growth factors such as transforming growth factor-β) probably occur at an early stage in the development toward hyperglycemia, glucose intolerance, and finally diabetes, at least in experimental models. This relationship between glycemia and fibrotic processes might explain why we observed an association between HbA1c and plaques with a relatively high content of fibrous tissue. However, to examine these questions properly, longitudinal studies are needed.

In addition to the variables we controlled for in Tables 2 and 3, adjustments for serum creatinine levels (reflecting atherosclerosis and glucose intolerance associated with renal failure) and elevated PTH (which is associated with insulin resistance and mild hyperglycemia) only had minor effects on the relationships found when included in the regression models.

The present study has some limitations, and our first concern is related to the possibility of bias. Our study group was large and had a high attendance rate (77% of the eligible subjects had their carotid arteries examined). However, severely ill or disabled individuals may have been underrepresented. Nevertheless, if this possible selection bias should invalidate our findings with respect to the risk of hard plaques, the prevalence of this plaque type would have to be very strongly associated with lower levels of HbA1c among the nonparticipants. We believe that this bias is unlikely and find it more plausible that nonparticipation may have weak-
enced the true relationship between HbA1c and plaque risk. One cannot, however, rule out the possibility that some of the declining prevalence of soft plaque with increasing HbA1c may be a result of survival and selection bias. It has recently been shown that soft stenotic plaque is associated with a higher risk of stroke than hard stenotic plaque, and hyperglycemic subjects who have developed soft plaque may therefore have suffered a stroke or died at an earlier age.

Recent epidemiological data suggest that postprandial hyperglycemia contributes to accelerated atherosclerosis and clinical cardiovascular events in diabetes. The DECODE study showed that abnormalities in 2-hour glucose concentrations after a 75-g glucose load were independent risk factors of mortality and that postchallenge plasma glucose and glycemic spikes were more strongly associated with increased IMT than fasting glucose or HbA1c level. Unfortunately, we did not measure postprandial glucose. However, postprandial glucose level is an important determinant of the HbA1c levels in nondiabetic persons. The relationship between glycemia and the formation of hard plaques could therefore reflect an association between daily postprandial glucose spikes and atherosclerotic processes in the arteries.

Because some medications used for treatment of hypertension may have adverse effects on glucose tolerance, we were concerned that these medications might have affected both glycemic control and the development of atherosclerosis. However, adjustments for present and previous use of antihypertensive medication and for cardiovascular diseases did not affect the relationships between HbA1c and plaques.

We conclude that increasing HbA1c levels are related to an increased risk of carotid artery plaques and that the relationship depends on plaque echogenicity. Our results suggest that the degree of glycemia contributes to the development of hard echogenic plaques and that the process takes place already at modestly elevated levels of HbA1c.

Acknowledgments

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References


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