Combined Effects of Hemoglobin A1c and C-Reactive Protein on the Progression of Subclinical Carotid Atherosclerosis
The INVADE Study
Dirk Sander, MD; Carla Schulze-Horn, MD; Horst Bickel, PhD; Hans Gnahn, MD; Eva Bartels, MD; Bastian Conrad, MD

Background and Purpose—Glycohemoglobin (hemoglobin A1c [HbA1c]) and high-sensitivity C-reactive protein (hsCRP) are risk indicators for atherosclerosis. Limited information exists regarding the combined effects of inflammation and hyperglycemia. We investigated the joint effects of both parameters on early carotid atherosclerosis progression and major vascular events in diabetic and nondiabetic subjects.

Methods—We analyzed the data of INVADE (Intervention Project on Cerebrovascular Diseases and Dementia in the Community of Ebersberg, Bavaria), a prospective, population-based study conducted in 3534 subjects (mean age, 69 years). In addition to common risk factors, measurements of carotid intima-media thickness (IMT), hsCRP, and HbA1c were performed at baseline and after 2 years of follow-up.

Results—For the entire population, IMT progression was significantly related to HbA1c (P=0.003) but not to hsCRP (P=0.06) after risk factor adjustment. The interaction hsCRP×HbA1c was highly significant (P=0.001), and the most pronounced IMT progression was seen in subjects with both parameters in the fourth quartiles compared with subjects with both parameters in the first quartiles (0.028 [0.025, 0.031] versus 0.012 mm/year [0.007, 0.019]; P=0.0013). We observed a significant joint effect of HbA1c and hsCRP on IMT progression in the diabetic (n=882) as well as the nondiabetic subgroup (n=2652). Subjects with HbA1c and hsCRP in the upper 2 quartiles had an increased risk for new vascular events (adjusted hazard ratio in diabetics: 4.3 [1.8, 7.3]; P=0.001; nondiabetics: 2.9 [1.6, 4.7]; P=0.001).

Conclusions—The combination of hyperglycemia and inflammation is associated with an advanced early carotid atherosclerosis progression and an increased risk of new vascular events in diabetic as well as nondiabetic subjects.

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Key Words: atherosclerosis ■ carotid arteries ■ diabetes mellitus ■ epidemiology ■ inflammation

Because a substantial part of incident myocardial infarction (MI) and stroke is unaccounted for by traditional cardiovascular risk factors, there is a great need to find novel and preferably modifiable factors that can identify subjects at high risk. Recent evidence suggests that high-sensitivity C-reactive protein (hsCRP) represents a powerful cardiovascular risk predictor, provides additive information on cardiovascular risk, and is positively associated with an enhanced atherosclerotic burden. Elevated glycohemoglobin (hemoglobin A1c [HbA1c]) is an established predictor for developing atherosclerosis beyond the risk associated with diagnosed diabetes and is independently associated with cardiovascular disease and total mortality in nondiabetes. Insights gained from the link between inflammation and hyperglycemia can yield predictive and prognostic information for further risk. The determination of carotid artery intima-media thickness (IMT) is a useful method for an easy, noninvasive evaluation of early carotid atherosclerosis and is independently related to the risk of stroke and cardiovascular events. Limited information exists regarding the joint effects of inflammation and hyperglycemia on the progression of early carotid atherosclerosis. We analyzed the combined effects of hsCRP and HbA1c on IMT progression in diabetic and nondiabetic subjects using a large population-based sample of the INVADE (Intervention Project on Cerebrovascular Diseases and Dementia in the Community of Ebersberg, Bavaria) study.

Methods
Subjects
This investigation is part of the INVADE study, a prospective and population-based cohort study in the elderly. All inhabitants of the community of Ebersberg, 30 km east of Munich, Germany, who were born before 1946 and were members of the health insurance company AOK (Allgemeine Ortskrankenkasse) were identified in the...
the AOK database and were then invited to participate (n=10 325). In the community of Ebersberg, >40% of all inhabitants aged >55 years were AOK members. During a baseline phase (2001–2003), 3905 subjects followed the invitation. A total of 3534 subjects were included in the present study. The remaining subjects were excluded because of incomplete laboratory data (n=195) or IMT data that were missing (n=95) or not analyzable (n=81). The baseline investigation was done by primary care physicians of the community of Ebersberg (n=65) and included a standardized questionnaire, a physical examination, evaluation of several risk factors, medical and disease history, a 12-lead ECG, and an overnight fasting venous blood sample for analysis in a central laboratory. A duplex ultrasonographic examination of the carotid arteries was done in all subjects according to a standardized protocol in 8 local centers of excellence after training. All data were entered into a central database after plausibility checks for further evaluation. After the initial baseline investigation, the primary care physician investigated the participants every 3 months. Complete follow-up investigations were scheduled after 2 years and were available for 3478 participants (98.5%). The local institutional review board approved this investigation. All patients provided informed consent before entering the study.

Cardiovascular Disease Status and Risk Factors
Follow-up information on current health status, medical history, lifestyle, cognitive status, mood disorders, drug use, and former cardiovascular risk factors was obtained by a computerized questionnaire at baseline. Risk factors determined included the following: body mass index (BMI) (kg/m²), smoking status, duration of smoking, alcohol consumption, actual medication, social status, education status, arterial hypertension (treatment with antihypertensive medication or documented blood pressure ≥140 mm Hg systolic or ≥90 mm Hg diastolic measured in a standardized fashion), diabetes mellitus (treatment with antidiabetic drugs or overnight fasting serum glucose levels ≥7.0 mmol/L or HbA₁c >6.0%), prevalent ischemic heart disease (documented by previous MI or angina pectoris, bypass surgery, or >50% angiographic stenosis of ≥1 major coronary artery), prevalent peripheral artery disease, and prevalent stroke (neurological deficit that persisted >24 hours, evaluated by a neurologist). MI¹⁰ and stroke¹¹ were diagnosed according to recent recommendations.

Clinical End Points
Once subjects enter the INVADE study, they are continuously monitored for major events through linkage of the study database with the occurrence of major adverse cardiovascular events, a composite of MI, stroke, and vascular death.

Laboratory Examinations
Overnight fasting blood samples were drawn from each subject and were transferred on ice to a central laboratory that performed all analyses. We used a high-sensitivity assay for measurement of serum hsCRP (N High Sensitivity CRP, DADE Behring) with a lower detection level of 0.175 mg/L and a coefficient of variation of 7.6%. The intra-assay precision ranges from 3.1% for a CRP content of 0.5 mg/L to 4.0% for a CRP content of 15 mg/L; the interassay precision was 2.5% and 2.6%, respectively.

HbA₁c was measured by high-pressure liquid chromatography separation of hemoglobin fractions with a reference value of 4.0% to 6.0% and a coefficient of variation of 1.8% on a Hi nAuto A1c HA-8140 device (KDK). In addition to these values, cholesterol, LDL, HDL, triglycerides, creatinine, fasting serum glucose, and homocysteine were measured.

Ultrasound Imaging
Eight experienced investigators performed the duplex ultrasonography using a standardized study protocol. The ultrasound data were stored on video or digital audiotapes, were transferred to the neurovascular laboratory of the Department of Neurology, and were digitalized if necessary. The measurements of mean common carotid artery IMT were performed as previously described in detail¹² with the use of a computer-supported image analysis system (Sigmascan-Pro 5.0, SPSS). The progression of early carotid atherosclerosis was defined as the difference between the last and first IMT measurement and was normalized as the change of IMT per year.

Statistical Analysis
All values are given as mean and 95% CI or as median and interquartile range (IQR) (range from the 25th to the 75th percentile) or as counts and percentages. We used χ² tests, independent t tests, Mann-Whitney U tests, and the Spearman rank correlation for univariate analysis, as appropriate. The study population was divided into quartiles according to levels of HbA₁c and hsCRP. The IMT differences between subgroups according to hsCRP and HbA₁c were tested with multiple linear regression techniques, and the covariate-adjusted mean IMT values (least square means) were reported. To
analyze the combined effect of HbA1c and hsCRP on IMT, the interaction term HbA1c×hsCRP was included in the regression models. In all models the IMT data were entered as continuous values. Survival curves were estimated by using the Kaplan-Meier product-limit method and compared by means of the log-rank test. Hazard ratios (HRs) were calculated with the Cox proportional hazard regression model. All multiple analyses were adjusted for the same relevant covariates (baseline age, sex, smoking status, cholesterol, LDL, BMI, systolic and diastolic blood pressure, prevalent ischemic heart disease, and statin use). Calculations were performed with JMP 5.01 software (SPSS Inc). A calculated difference of P<0.05 was considered statistically significant.

Results
The mean age of the participants was 69 (68.8, 69.4) years; 1446 (41%) were male. Diabetes mellitus was diagnosed in 882 patients (25%). Of these, 662 had known diabetes at baseline; 305 patients (41%) were male. Diabetes mellitus was diagnosed in 882 patients (25%).

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Effects of hsCRP and HbA1c on IMT Progression
There was a significant univariate association between IMT progression and HbA1c (r=0.08; P<0.0001) as well as hsCRP (r=0.06; P<0.0001). Analyzing the effects of HbA1c and CRP after adjusting for the other risk factors, we found a significant overall relationship between HbA1c, hsCRP, and IMT progression (F=3.53; P=0.0001; Figure 1). However, after risk factor adjustment only HbA1c (P=0.003) but not hsCRP (P=0.06) remained significantly associated with IMT progression. The interaction hsCRP×HbA1c was highly significant (F=7.36; P=0.001). Accordingly, the most pronounced IMT progression was seen in subjects with both parameters in the fourth quartiles. In contrast, subjects with both parameters in the first quartiles revealed the lowest IMT progression (0.028 [0.025, 0.031] versus 0.012 mm/year [0.007, 0.019]; P=0.0013; Figure 1).

Diabetic Versus Nondiabetic Subjects
Baseline data of both subgroups are given in Table 1. Diabetic subjects showed significantly higher hsCRP values, increased baseline IMT, and enhanced IMT progression.
progression remained significantly related to HbA1c in both diabetic subjects (Table 1). After risk factor adjustment, IMT predictor of IMT progression after adjustment (Table 2). In diabetic subjects, hsCRP was a highly significant in both subgroups (Table 2), indicating that HbA1c and hsCRP jointly contribute to IMT progression. The interaction term HbA1c P = 0.0001 and P = 0.0007; Table 2). The interaction term HbA1c [in quartiles] × hsCRP [in quartiles] to the multivariate model. Adding this term, we observed a significant change of 8.82; df 2; P = 0.01). To analyze the combined effects of hsCRP and HbA1c in more detail, the subject population was then further

### Effects of hsCRP and HbA1c on Major Cardiovascular Adverse Events

During follow-up, 143 cardiovascular events occurred in 139 patients (4%), including 62 MIs, 68 nonfatal strokes, and 13 vascular deaths. Adjusted HRs for the occurrence of cardiovascular events according to increasing quartiles of HbA1c and hsCRP are given in Table 3 for the diabetic and nondiabetic subgroups. We then tested for interaction between HbA1c and hsCRP with cardiovascular events. Interactions were considered by adding an interaction term (HbA1c [in quartiles] × hsCRP [in quartiles]) to the multivariate model. Adding this term, we observed a significant change of the model fit as indicated by a likelihood ratio test (χ² = 8.82; df 2; P = 0.01). To analyze the combined effects of hsCRP and HbA1c in more detail, the subject population was then further

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**TABLE 2. Determinants of Common Carotid Artery IMT as Evaluated by Multiple Regression**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Nondiabetic Patients (n=2652)</th>
<th>Diabetic Patients (n=882)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β</td>
<td>95% CI of β</td>
</tr>
<tr>
<td>HbA1c, %</td>
<td>0.16</td>
<td>0.13 to 0.21</td>
</tr>
<tr>
<td>hsCRP, mg/L</td>
<td>0.021</td>
<td>−0.01 to 0.053</td>
</tr>
<tr>
<td>HbA1c × hsCRP</td>
<td>0.19</td>
<td>0.12 to 0.26</td>
</tr>
<tr>
<td>Age, y</td>
<td>0.15</td>
<td>0.09 to 0.35</td>
</tr>
<tr>
<td>Female sex</td>
<td>−0.11</td>
<td>−0.18 to −0.06</td>
</tr>
<tr>
<td>Systolic BP, mm Hg</td>
<td>0.17</td>
<td>0.05 to 0.28</td>
</tr>
<tr>
<td>Diastolic BP, mm Hg</td>
<td>0.19</td>
<td>0.04 to 0.31</td>
</tr>
<tr>
<td>Pack-years</td>
<td>0.08</td>
<td>0.05 to 0.11</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>0.05</td>
<td>0.021 to 0.089</td>
</tr>
<tr>
<td>Cholesterol, mmol/L</td>
<td>0.015</td>
<td>−0.016 to 0.033</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/L</td>
<td>0.068</td>
<td>0.011 to 0.099</td>
</tr>
<tr>
<td>Prevalent ischemic heart disease</td>
<td>0.004</td>
<td>−0.009 to 0.018</td>
</tr>
<tr>
<td>Statin administration</td>
<td>−0.03</td>
<td>−0.17 to 0.04</td>
</tr>
</tbody>
</table>

BP indicates blood pressure. HbA1c and hsCRP were entered as continuous values into the model.

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**TABLE 3. HRs and 95% CIs for Major Vascular Adverse Events in Diabetic and Nondiabetic Patients According to hsCRP and HbA1c Calculated by Multivariate Cox Proportional Hazard Analysis**

<table>
<thead>
<tr>
<th></th>
<th>Laboratory Range</th>
<th>HR (95% CI) Adjusted*</th>
<th>P</th>
<th></th>
<th>Laboratory Range</th>
<th>HR (95% CI) Adjusted*</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nondiabetic Patients (n=2652)</td>
<td></td>
<td></td>
<td></td>
<td>Diabetic Patients (n=882)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>hsCRP quartile 1</td>
<td>&lt;0.9 mg/L</td>
<td>1.0</td>
<td>...</td>
<td>&lt;1.3 mg/L</td>
<td>1.0</td>
<td>...</td>
<td></td>
</tr>
<tr>
<td>hsCRP quartile 2</td>
<td>0.9 to 1.9 mg/L</td>
<td>1.22 (0.78, 1.95)</td>
<td>0.1</td>
<td>1.3 to 2.5 mg/L</td>
<td>1.55 (0.52, 2.33)</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>hsCRP quartile 3</td>
<td>1.9 to 3.4 mg/L</td>
<td>1.55 (1.22, 2.09)</td>
<td>0.009</td>
<td>2.5 to 4.1 mg/L</td>
<td>1.95 (1.18, 3.45)</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>hsCRP quartile 4</td>
<td>&gt;3.4 mg/L</td>
<td>1.97 (1.44, 3.01)</td>
<td>0.005</td>
<td>&gt;4.1 mg/L</td>
<td>2.77 (1.51, 6.2)</td>
<td>0.006</td>
<td></td>
</tr>
<tr>
<td>HbA1c quartile 1</td>
<td>&lt;5.3%</td>
<td>1.0</td>
<td>...</td>
<td>&lt;5.9%</td>
<td>1.0</td>
<td>...</td>
<td></td>
</tr>
<tr>
<td>HbA1c quartile 2</td>
<td>5.3% to 5.5%</td>
<td>1.03 (0.77, 1.65)</td>
<td>0.8</td>
<td>5.9% to 6.3%</td>
<td>1.34 (0.98, 2.12)</td>
<td>0.09</td>
<td></td>
</tr>
<tr>
<td>HbA1c quartile 3</td>
<td>5.5% to 5.8%</td>
<td>1.26 (0.99, 1.98)</td>
<td>0.05</td>
<td>6.3% to 7%</td>
<td>1.82 (1.22, 4.32)</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>HbA1c quartile 4</td>
<td>&gt;5.8%</td>
<td>1.44 (1.07, 2.33)</td>
<td>0.02</td>
<td>&gt;7%</td>
<td>2.01 (1.35, 5.32)</td>
<td>0.009</td>
<td></td>
</tr>
<tr>
<td>Group I</td>
<td>†</td>
<td>1.0</td>
<td>...</td>
<td>†</td>
<td>1.0</td>
<td>...</td>
<td></td>
</tr>
<tr>
<td>Group II</td>
<td>†</td>
<td>1.77 (1.21, 2.88)</td>
<td>0.006</td>
<td>†</td>
<td>2.3 (1.1, 5.54)</td>
<td>0.008</td>
<td></td>
</tr>
<tr>
<td>Group III</td>
<td>†</td>
<td>2.93 (1.55, 4.74)</td>
<td>0.001</td>
<td>†</td>
<td>4.3 (1.8, 7.3)</td>
<td>0.001</td>
<td></td>
</tr>
</tbody>
</table>

*Adjusted for baseline age, sex, smoking status, cholesterol, LDL, BMI, systolic and diastolic blood pressure, prevalent ischemic heart disease, and statin use. †Group I included subjects with both hsCRP and HbA1c in the lower 2 quartiles; group II, subjects with 1 parameter in the upper and the other parameter in the lower 2 quartiles; and group III, subjects with both parameters in the upper 2 quartiles.
subdivided into 3 groups: group I (n=949, 26.9%) consisted of subjects with both hsCRP and HbA1c in the lower 2 quartiles; group II (n=1586, 44.9%) consisted of subjects with 1 of the parameters in the lower 2 quartiles and the other parameter in the upper 2 quartiles; and group III (n=999, 28.2%) consisted of subjects with both parameters in the upper 2 quartiles. A gradually increased risk for adverse events from group I to III was observed (Figure 2). Adjusted HRs for the occurrence of vascular events were most increased in subjects with both parameters in the upper 2 quartiles (Table 3), and diabetic subjects in particular showed a substantially increased risk for new vascular events (HR=4.3; Table 3).

Discussion
This investigation demonstrates several important findings that may have implications for therapeutic approaches and future studies. First, HbA1c and hsCRP are differently related to carotid atherosclerosis in diabetic compared with nondiabetic subjects. In nondiabetics, we detected a significant association between IMT progression and HbA1c, whereas hsCRP was not significantly related to progression of IMT after adjustment for several conventional risk factors. In contrast, in diabetic patients HbA1c and hsCRP are independent predictors of IMT progression.

Until now there have been conflicting findings regarding the relationship between hsCRP and IMT. Some cross-sectional studies demonstrated that hsCRP is related to the extent of IMT, whereas other prospective studies could not establish a significant association with IMT progression after adjustment for several traditional risk indices. An analysis from the Rotterdam Study has shown that hsCRP predicts progression of more advanced atherosclerosis with the use of a composite plaque score. Additionally, a recent small investigation also detected a relationship between progression of plaque number as well as plaque score and CRP. In summary, these data indicate that hsCRP may play a more important role in advanced stages of atherosclerosis or active atherosclerotic disease rather than in early atherosclerosis as studied by use of IMT measurements.

Previous cross-sectional studies could demonstrate a positive relation between increasing levels of HbA1c and carotid IMT. Data from the Tromsø study indicated that even modestly elevated levels of glycohemoglobin are strongly related to the prevalence of carotid plaques with high echogenicity in nondiabetic individuals. To the best of our knowledge, the present study is the first large and population-based investigation that prospectively demonstrated a significantly enhanced IMT progression in subjects with average HbA1c values in the upper normal range as well as in the nondiabetic subgroup. This pronounced effect of HbA1c on early carotid atherosclerosis progression corroborates the hypothesis that HbA1c may be causally related to atherosclerosis even at low levels and may explain the positive association between HbA1c and future cardiovascular risk even in nondiabetic subjects.

The most important result of our study is that even slightly elevated levels of hsCRP and HbA1c jointly contribute to the progression of atherosclerosis at early and subclinical stages of the disease in diabetic as well as nondiabetic subjects. In the nondiabetic subgroup, median HbA1c levels were 5.5% and median hsCRP levels were 1.8 mg/L are jointly related to significantly enhanced IMT progression and were associated with a nearly 3-fold increased risk for new major vascular events. The combined effect of both parameters on vascular events is even more pronounced in diabetic subjects with a HR of 4.3. Inflammation is one of the primary mechanisms of atherogenesis, and elevation of hsCRP heralds atherothrombotic events. However, hsCRP was described not merely as a marker of atherosclerosis risk but also as directly promoting endothelial cell activation, adhesion molecule expression, and resultant dysfunction. Our data further suggest that in the presence of hyperglycemia, hsCRP exerts harmful effects on
atherosclerosis progression, particularly in diabetic subjects, even at subclinical stages. Although an association between hsCRP, hyperglycemia, and atherosclerosis has been described in clinical and experimental observations, the mechanisms through which hyperglycemia might strengthen the proatherogenic effects of hsCRP are not unequivocally clarified. Several findings suggest that hsCRP may be related to atherogenesis by diminishing nitric oxide. Interestingly, acute hyperglycemia also reduces nitric oxide bioavailability, pointing to the possibility that the combined effect of raised hsCRP concentrations and increased HbA1c may jointly reduce nitric oxide bioavailability.

Because a substantial part of incident MI and stroke is unaccounted for by traditional cardiovascular risk factors, there is a great need to find novel and preferably modifiable factors that can identify subjects at high risk. Considering the combined effect of HbA1c and hsCRP may help to more exactly identify and better treat these patients at high risk. Our data show that risk estimates for the progression of atherosclerosis associated with both HbA1c and CRP in the upper quartiles were at least as high as those associated with traditional risk factors and clearly indicate an increased risk for new major vascular events even in asymptomatic nondiabetic subjects. Because the risk was even greater in diabetic subjects, these findings underscore the importance of optimized diabetes control particular in diabetic subjects with elevated hsCRP levels. Recent findings demonstrate a regression of carotid atherosclerosis by control of postprandial hyperglycemia in type 2 diabetes and a joint effect of elevated CRP and HbA1c levels on poor outcome because of cardiovascular adverse events in patients with advanced atherosclerotic disease. It is obvious to suggest that treatment with statins targeting chronic inflammation may be particularly beneficial for these subjects. Furthermore, our data may explain the beneficial effects of statin therapy on clinical end points, particularly in diabetic patients, as recently demonstrated in the Heart Protection Study and Collaborative Atorvastatin Diabetes Study.

Our study has some limitations. The follow-up period of 2 years is relatively short, and the number of vascular events is therefore limited. However, the major strength of our study is the nearly complete follow-up (98.4%) and the fact that we were able to examine accepted surrogate markers (IMT) during follow-up in 3478 of 3534 of our subjects.

In conclusion, the combined occurrence of elevated HbA1c, indicating hyperglycemia, and elevated hsCRP, indicating low-level inflammation, is independently associated with an advanced subclinical carotid atherosclerosis progression and an increased incidence of new vascular events.

Acknowledgments

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References


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