Original Article

The Hemoglobin Glycation Index Is Not an Independent Predictor of the Risk of Microvascular Complications in the Diabetes Control and Complications Trial

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The Diabetes Control and Complications Trial (DCCT) demonstrated that intensive therapy aimed at improved glucose control markedly reduced the risk of diabetes complications compared with conventional therapy. The principal determinant of risk was the history of glycemia. Recently, McCarter et al. (*Diabetes Care* 27:1259–1264, 2004) have presented analyses of the publicly available DCCT data using their hemoglobin glycation index (HGI), which is computed as the difference between the observed HbA1c (A1C) and that predicted from the level of blood glucose. In their analyses, the HGI level was a significant predictor of progression of retinopathy and nephropathy in the DCCT, which the authors claimed to support the hypothesis that the biological propensity for glycation, so-called biological variation in glycation, is another mechanism that determines risk of complications. However, we have criticized these analyses and conclusions because, from statistical principles, the glycation index must be positively correlated with the A1C level and thus may simply be a surrogate for A1C. Herein, we present the statistical properties of the glycation index to document its high correlation with A1C. We then replicate the analyses of McCarter et al. using both the HGI and the A1C together. Analyses show conclusively that the glycation index is not an independent risk factor for microvascular complications and that the effect of the glycation index on risk is wholly explained by the associated level of A1C. The HGI should not be used to estimate risk of complications or to guide therapy. *Diabetes* 56:1913–1921, 2007

The Diabetes Control and Complications Trial (DCCT) conclusively showed that intensive therapy, aimed at maintaining glycemic levels as close to the nondiabetic range as possible, when compared with conventional therapy with no numeric glucose goals, markedly reduced the onset and progression of diabetic retinopathy, nephropathy, and neuropathy (1). Subsequent epidemiologic analysis showed that the principal determinant of the risk of complications in each treatment group was the lifetime history of glycemia as represented by the level of baseline HbA1c (A1C) and duration of diabetes at study entry, and by the mean level of A1C during the study and the length of study treatment (2), the latter dependent on when the subject entered the study. Moreover, these relationships were characterized by a smooth risk gradient with a 44% reduction in risk of retinopathy progression per 10% lower A1C, with no difference in the relationship between glycemia and complications for intensive and conventional treatment groups, and no breakpoint or threshold in the relationship above the nondiabetic range (3).

Recently, McCarter et al. (4) have presented analyses of the publicly available DCCT data that have suggested a different mechanism for the development of complications, specifically the individual propensity for glycation as measured by the hemoglobin glycation index (HGI) (5). This index was defined as the difference between the measured level of A1C and the level that would be predicted from the statistical relationship between A1C and blood glucose. The hypothesis is that individuals with a high glycation index will have a propensity to glycate other proteins and macromolecules leading to a higher risk of developing microvascular complications compared with individuals with average or low glycation indexes. The authors concluded that the biological variation in the propensity to glycate hemoglobin is as important, if not more so, than is the level of A1C.

We have previously criticized these analyses and this interpretation of the DCCT data (6) by showing statistically that the glycation index must be correlated with A1C. Thus the glycation index may simply be a surrogate for A1C, and the association between the glycation index and microvascular complications may largely be explained by its correlation with A1C. Herein, we present a brief explanation of the statistical properties of the glycation index and present further analyses of the DCCT data to show that the glycation index is not an independent predictor of retinopathy or neuropathy in the DCCT when adjusted for the level of A1C.

RESEARCH DESIGN AND METHODS

The design and methods of the DCCT have been extensively documented elsewhere (1). Briefly, the DCCT cohort consisted of 1,441 subjects with type 1 diabetes, of whom 711 were randomly assigned to intensive therapy aimed at maintaining glycemic levels as close to the nondiabetic range as possible. The 730 patients assigned to conventional therapy had the goal of maintaining

clinical well-being with no specific glucose targets. A1C levels assessed monthly in the intensive group were unmasked to patients and physicians, whereas values assessed quarterly in the conventional group were masked.

Two cohorts of subjects were enrolled. The primary prevention cohort of 726 patients had 1–5 years of diabetes with no retinopathy and albumin excretion rate (AER) <40 mg/24 h on entry. The secondary intervention cohort of 715 patients had 1–15 years of diabetes with minimal to moderate retinopathy and AER <200 mg/24 h on entry.

Severity of retinopathy was assessed by fundus photographs obtained every 6 months that were centrally graded according to the Early Treatment Diabetic Retinopathy Study (7) scale of severity. Nephropathy was assessed by an annual timed renal collection for measurement of the AER.

As used by McCarter et al. (4), retinopathy progression was defined as a sustained progression of three or more steps from the level at baseline, confirmed on two successive 6-monthly visits. Advanced microalbuminuria (progression of nephropathy) was defined as observation of an AER ≥100 mg/24 h among those with AER <100 mg/24 h at baseline, or observation of an AER ≥300 among subjects in the secondary cohort.

Subjects in both treatment groups conducted self-monitoring of blood glucose, with greater frequency in the intensive group. The daily levels were used for diabetes management and were not recorded centrally for data analysis. However, each subject was requested to obtain every 3 months a seven-point profile of blood glucose values before and 90 min after each meal and at bedtime. The blood was collected in Profilsets, and the hemolysates were analyzed for glucose in the DCCT Central Biochemistry Laboratory. The mean blood glucose (MBG) was computed from the profile measures.

As in previous analyses (2), the updated or current mean A1C was computed as the mean of all quarterly A1C values since entry up to and including the A1C at each visit. For example, the updated mean at month 12 for each subject is the mean of the values at months 3, 6, 9, and 12.

We attempted to replicate the analyses in McCarter et al. (4) as precisely as possible from the published description of their methods and personal correspondence with the authors. However, the programs and data used by McCarter et al. (4) were lost (personal communication, R. McCarter). Thus the dataset used herein is not exactly the same as that used by McCarter et al. (4).

HGI. The HGI was computed using methods as described by McCarter et al. (4). In a longitudinal regression model for repeated measures (8), the A1C at each quarterly visit was regressed on the Profilset MBG collected closest to the day of the A1C collection. The model also adjusted for age, sex, DCCT treatment group, duration of diabetes at baseline, primary intervention versus secondary prevention cohort, and race. For each visit of a subject, the difference between the observed A1C and the A1C predicted from the MBG and other covariates in the regression model, i.e., the residual, was computed. These residuals were averaged over all visits for each subject to obtain the HGI value for that subject. Subjects were ranked according to their HGI values and categorized into three groups (low, moderate, and high HGI) according to the tertiles of the distribution of HGI values. Of the 1,441 randomized subjects, 1,392 had Profilset blood glucose values and fundus photographs measured after randomization, and 1,390 had AER measurements.

Proportional hazards models. The discrete logistic Cox proportional hazards regression model (9) adjusted for tied event times was used to assess the effect of HGI group (high, moderate, or low) on the risk of retinopathy or nephropathy progression, adjusting for other baseline covariates. The initial models were identical to those used by McCarter et al. (4). Models were then re-fit to assess the effect of HGI group with adjustment for the time-dependent current updated mean A1C.

The Breslow estimate of the background hazard function (9) was used in conjunction with the proportional hazards model–based coefficient estimates to provide an estimate of the cumulative hazard function and the corresponding cumulative incidence function using S-Plus version 7.0 (Insightful, Seattle, WA). The resulting cumulative incidence of each complication, adjusted for other covariates, was then plotted. All other analyses were performed using SAS version 8.2 (SAS Institute, Cary, NC). Effects nominally significant at $P \leq 0.05$ are cited.

RESULTS

Statistical properties of the glycation index. The HGI value for a subject at a given visit is defined (4,5) as the difference between the observed level of A1C at that point in time versus that predicted from the linear regression equation using the MBG (and other factors). The mean HGI value for a given subject is the mean of the index values over all of that subject's visits.

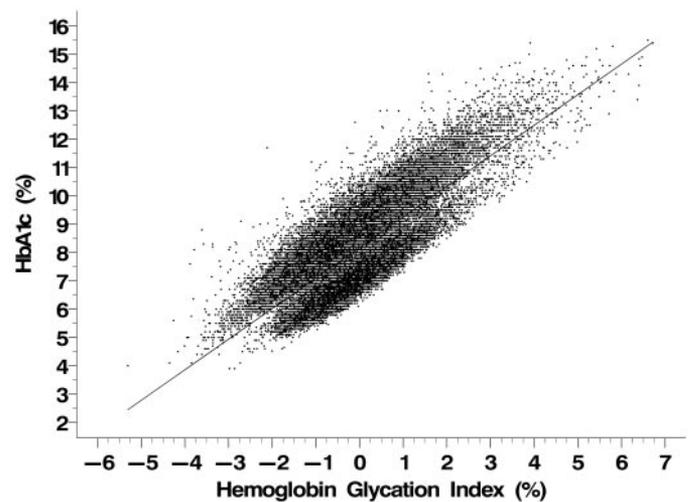Consider a single subject. Let H_j denote the level of A1C

FIG. 1. Scatter plot of the A1C versus HGI for all visits of all DCCT subjects, with the estimated regression line; intercept 8.2, slope 1.08 A1C % increase per unit increase in the HGI, and correlation of 0.77.

at the jth visit for that subject, and likewise let G_j denote the within-day MBG from the Profilset at that visit, where $j = 1, 2, \ldots, J$ denotes the successive visits for a subject with J visits in total. For a subject with 6.5 years of follow-up (the study average), then $J = 26$ quarterly visits. The expected level of A1C at a visit (h_j), given the observed level of glucose, is then estimated from the linear regression equation with intercept a and slope b

$$h_j = a + bG_j \tag{1}$$

The residual (E_j) is computed as the difference between the level of A1C observed and that predicted from the model:

$$\begin{aligned} E_j &= H_j - h_j \\ &= H_j - a - bG_j \end{aligned} \tag{2}$$

Thus, the residual at a visit is a simple linear function of both the observed level of A1C and the level of glucose at that visit.

The HGI value at the jth visit for a subject is computed in a similar manner but as the residual from a regression model that includes other factors in addition to the MBG at each visit. Figure 1 presents a scatterplot of the A1C and the HGI over all visits of all subjects with the estimated regression line. The correlation is 0.77, and the slope of the relationship is 1.08. For a unit (%) difference in the HGI between subjects at a visit, on average there is a difference of 1.08% in the mean A1C at that visit. Thus the HGI and the A1C at each visit are strongly related.

In the analyses by McCarter et al. (4), the HGI value for a subject with J visits is defined as the mean of the HGI values (residuals) over all J visits for that subject, thus providing a single measure of the propensity for glycation for that subject. From Eq. 2, the mean HGI for a subject is also a function of the mean A1C over all visits (M_H) and the mean glucose (M_G) over all visits for that subject, i.e., the HGI is proportional to $M_H - a - bM_G$. Thus, the mean HGI is strongly related to the mean A1C (M_H), with a 0.73 correlation among the DCCT subjects.

Thus, if subjects are divided into categories such as high, moderate, and low levels of the HGI, as in the studies of McCarter et al. (4) and Hempe et al. (5), the mean levels of A1C will also tend to be so ordered. Accordingly, any

TABLE 1
DCCT baseline characteristics by treatment group

	Conventional	Intensive
n	729	710
Cohort		
Secondary intervention	377 ± 51.7	348 ± 49.0
Primary prevention	352 ± 48.3	362 ± 51.0
Age (years)	26.5 ± 7.1	27.1 ± 7.1
Race (Caucasian)	703 ± 96.4	686 ± 96.6
Sex (male)	394 ± 54.0	365 ± 51.4
Duration of type 1 diabetes (years)	5.7 ± 4.1	6.0 ± 4.2
A1C at eligibility (%)	9.05 ± 1.63	9.08 ± 1.59
MBG at DCCT baseline (mmol/l)	12.8 ± 4.4	13.0 ± 4.6

Data are n (%) and means ± SD. $P > 0.10$ for all comparisons.

association between the HGI and risk of complications could be solely due to the relationship between the risks of complications with the level of A1C mediated through the correlation of the HGI with the A1C.

In this report, we replicate all of the analyses presented by McCarter et al. (4) and then conduct an additional analysis using a statistical adjustment for the A1C to determine whether the HGI is an independent risk factor

for microvascular complications in the DCCT above and beyond the A1C.

Regression models using the HGI and A1C. Table 1 shows the distributions of the baseline characteristics used in these analyses, with no differences between groups.

Figure 2 shows the regression of the A1C on the MBG over all visits for all participants and for those subjects within the low, moderate, and high HGI categories, as previously presented by McCarter et al. (4). To these figures, we have added a horizontal reference line to indicate the mean A1C among all visits. Table 2 presents the actual mean values of the MBG, HGI, and A1C within each category. All three quantities increased when comparing the low, moderate, and high HGI groups. The mean A1C was 7.25% among those in the low HGI group versus 7.85 among those in the moderate group and 9.34 in the high group.

Figure 3A presents the cumulative incidence of retinopathy among those in the high, moderate, and low HGI groups, as shown in McCarter et al. (4), without adjustment for A1C. Those in the high HGI group have a risk 5.3-fold greater than that of those in the low HGI group, and those in the moderate HGI group have a relative risk of 2.6 versus the low HGI group (both $P < 0.0001$). The
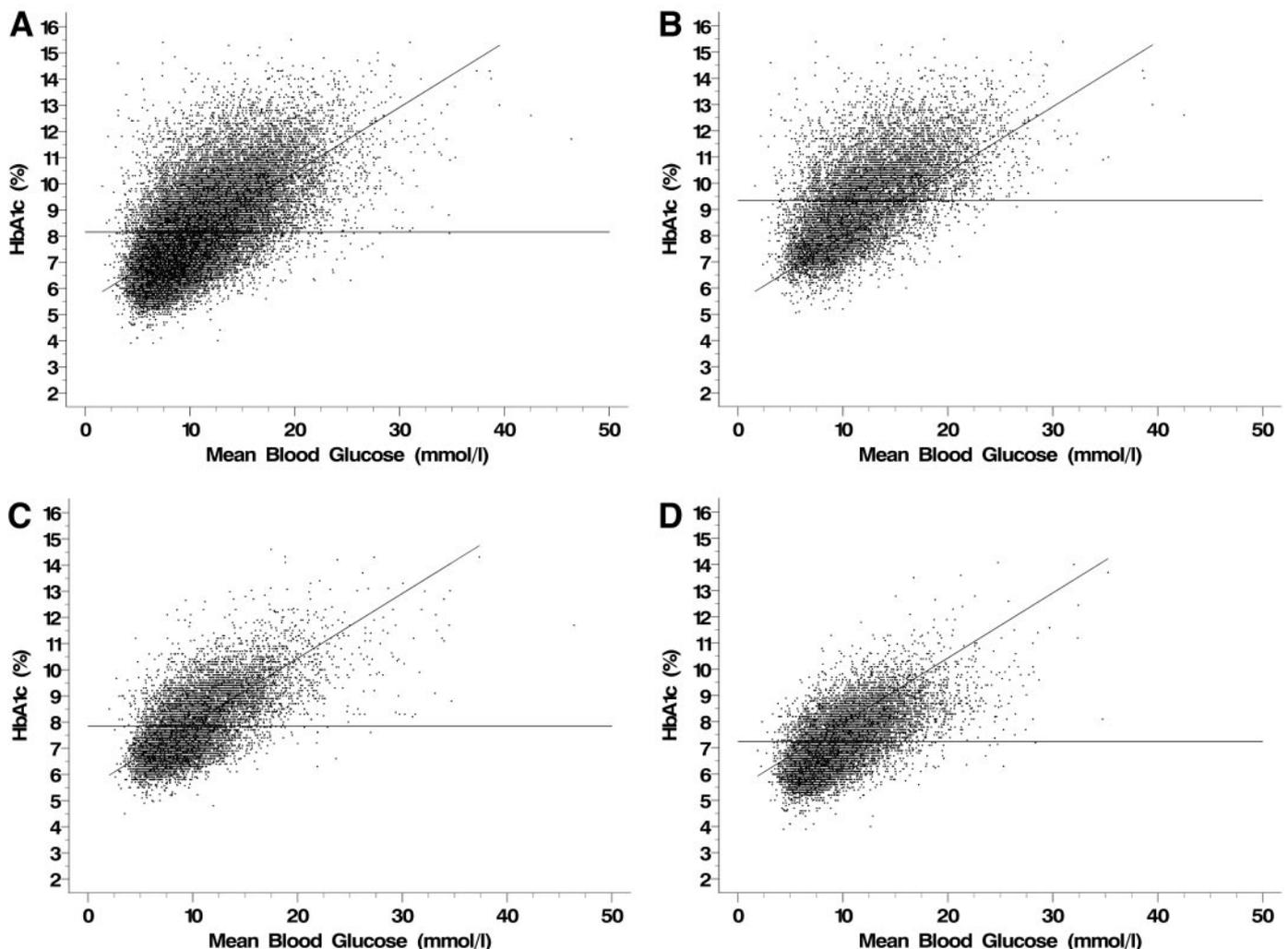

FIG. 2. Scatter plots of the observed A1C versus the observed within-profile MBG at all visits during the DCCT. *A*: For all participants. *B–D*: For those participants in the high, moderate, and low HGI groups, respectively. The regression line shown in each panel was derived from the simple regression of A1C on the MBG from the population. The horizontal line is the mean A1C in each panel.

overall effect of the HGI among all three categories is highly significant ($P < 0.0001$ on 2 degrees of freedom [df]). Figure 3B then shows the cumulative incidence within each of these groups after adjusting for the current updated mean A1C. When HGI group is added to the model with A1C, neither the high versus low nor moderate versus low HGI group pairwise comparisons had a relative risk that is significantly different from unity, and the overall effect of the HGI is no longer significant ($P = 0.50$ on 2 df).

Likewise, Fig. 4A presents the cumulative incidence of nephropathy (advanced microalbuminuria) for each HGI group without adjustment for A1C that also shows a highly significant difference among groups. However, when the effect of the HGI is adjusted for the effect of the updated mean A1C (Fig. 4B), the effect of the HGI is no longer significant.

McCarter et al. (4) also conducted an analysis comparing risk of retinopathy in the HGI categories among subjects in the lower and upper thirds of the distribution of MBG values (the low and high MBG groups). Figure 5A and B presents the analyses within the low MBG group without and with adjustment for the mean A1C, and Fig. 6A and B likewise presents those within the high MBG group. After adjustment for A1C, there are no differences between the HGI groups.

DISCUSSION

The HGI is computed as the mean difference (or mean residual) between the observed level of A1C and that predicted from its regression on the MBG and other baseline factors. From the statistical properties of such computed residuals, it is readily shown that the HGI must be strongly correlated with the mean A1C.

In response to our prior critique (6), Chalew et al. (10) dismissed the characterization of the HGI as a residual, because the value assigned to each subject "represents a measure of each individual's average directional divergence in A1C from that predicted over dozens of quarterly measurements; it is not merely a single regression residual." In other words, the authors' HGI is an average of such residuals. Taking the average of these residuals does not change the fundamental properties of the HGI. Their summary HGI computed for each patient had a correlation of 0.73 with the each patient's mean A1C over the period of the DCCT, which begs the question as to whether it is the level of A1C or the level of HGI that is most strongly associated with the risk of complications.

Prior analyses (2,3) have shown that the current updated mean A1C over time is the principal determinant of the risk of microvascular complications in the DCCT. Thus, as stated in our prior critique (6), it follows that the glycation index is a surrogate for A1C, and it is a statistical tautology that the HGI will also be associated with risk. The only way to determine whether it is the HGI per se or its correlation with A1C that explains the association between the HGI and risk of complications is to conduct analyses with both variables together. In response to our prior critique (6), Chalew et al. (10) claimed that such an approach would be undesirable "since A1C is highly correlated with MBG." In fact, as shown herein, the correlation between the A1C and MBG is $r = 0.65$, which falls far below the level ($r > 0.95$) at which a collinearity might be introduced.

Thus, the analyses presented herein take the debate one step further to evaluate whether the purported propensity to glycation as measured by the HGI is an independent risk factor for retinopathy and nephropathy after adjusting for the level of A1C. Analyses herein show conclusively that it is the level of A1C and not the HGI that determines risk of complications. Thus, the association between the HGI and risk of complications previously reported by McCarter et al. (4) is in fact wholly due to its intercorrelation with the A1C, and the HGI has no effect on risk after adjustment for the level of A1C.

A similar index of discordance between the observed level of A1C and other measures of glycemic control, specifically fructosamine, termed the glycosylation gap, has been described by Cohen et al. (11). The glycosylation gap is similarly defined as the difference between the observed A1C and the level predicted from its regression on fructosamine. This measure was shown to be associated with levels of nephropathy in a cross-sectional sample of subjects with diabetes. In their small sample of 40 subjects with a mean of 30 years of type 1 diabetes, the level of A1C alone was not associated with the level of nephropathy, whereas the glycosylation gap had a significant association, even after adjusting for the level of A1C. The fact that only a single A1C was obtained and that it was not associated with the level of nephropathy calls into question the meaning of these results.

Although the HGI, as computed from the DCCT data, may not be an independent predictor of risk of microvascular complications, the DCCT data clearly show that for a given level of MBG, there is substantial variation in the level of A1C among patients. McCarter et al. (4) based their analyses on the conjecture that this "biological variation" in the level of A1C relative to the level of blood glucose can be ascribed to a variation in a theoretical biological index of glycation among subjects (4), whereby individuals with the same mean level of blood glucose could glycate (or deglycate) proteins at different rates (12,13). An actual biological mechanism accounting for such theoretical inter-individual differences has not been demonstrated.

There are other possible explanations for the observed variation in A1C relative to the level of blood glucose. The most obvious potential explanation is that quarterly measurement of the seven-point glucose profiles in the DCCT did not capture the actual mean levels of glucose, or those components of the glucose profile, over a period of weeks

TABLE 2
MBG, HGI, and A1C among all visits for subjects classified by low, moderate, and high levels of the HGI

HGI group	n (subjects)	n (Obs)	MBG	HGI	A1C
Low (<-0.425)	467	11,338	10.00 (3.75)	−0.91 (0.78)	7.25 (1.17)
Moderate (-0.425 to 0.249)	470	11,351	10.17 (3.99)	−0.09 (0.75)	7.85 (1.29)
High (>0.25)	502	11,350	12.20 (4.68)	0.92 (1.12)	9.34 (1.66)

Data are means (SD). Obs, quarterly visit values.

A

RR(High vs. Low)=5.33
P<0.0001
RR(Moderate vs. Low)=2.64
P<0.0001
Overall 2df test χ^2=82.48
P<0.0001

High HGI

Moderate HGI

Low HGI

B

Low HGI
Moderate HGI
High HGI

RR(High vs. Low)=0.94
P=0.8121
RR(Moderate vs. Low)=1.15
P=0.5289
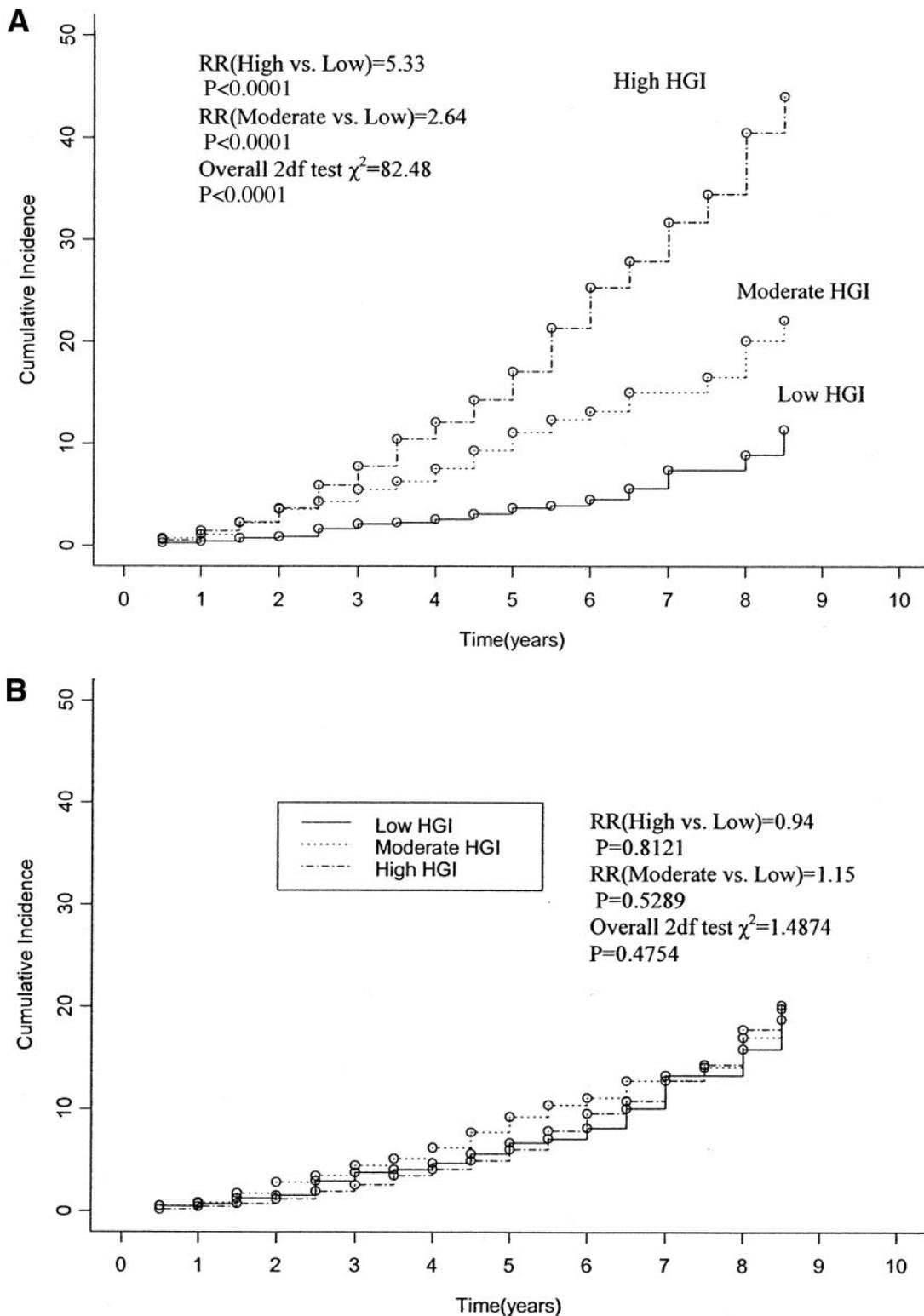Overall 2df test χ^2=1.4874
P=0.4754

FIG. 3. Cumulative incidence of retinopathy progression within the high, moderate, and low HGI groups obtained from a Cox proportional hazards regression model adjusted for the effects of age, diabetes duration, sex, treatment group, cohort, and MBG as a time-dependent covariate. The adjusted relative risk (RR) for moderate versus low HGI and for high versus low HGI with *P* value and the overall 2-df Wald test, χ^2, and *P* values are shown. *A*: Without adjustment for A1C. *B*: With adjustment for the A1C at eligibility and the time-dependent current updated mean A1C.

that in turn determine the observed value of A1C, with sampling error also a distinct possibility. Furthermore, in the DCCT, the correlation between the MBG and A1C at a visit was only 0.65, suggesting that the quarterly profile set provided weak information regarding the relationship between the A1C and the values of blood glucose over the

past several weeks. Of note, two previous studies have demonstrated much higher correlations ($r = 0.95$ or 0.96) between mean glucose levels, calculated from frequent daily glucose measures over 4 to 8 weeks, and a single A1C measured at the end of the study period (14,15). Although the relatively small number of study subjects in these
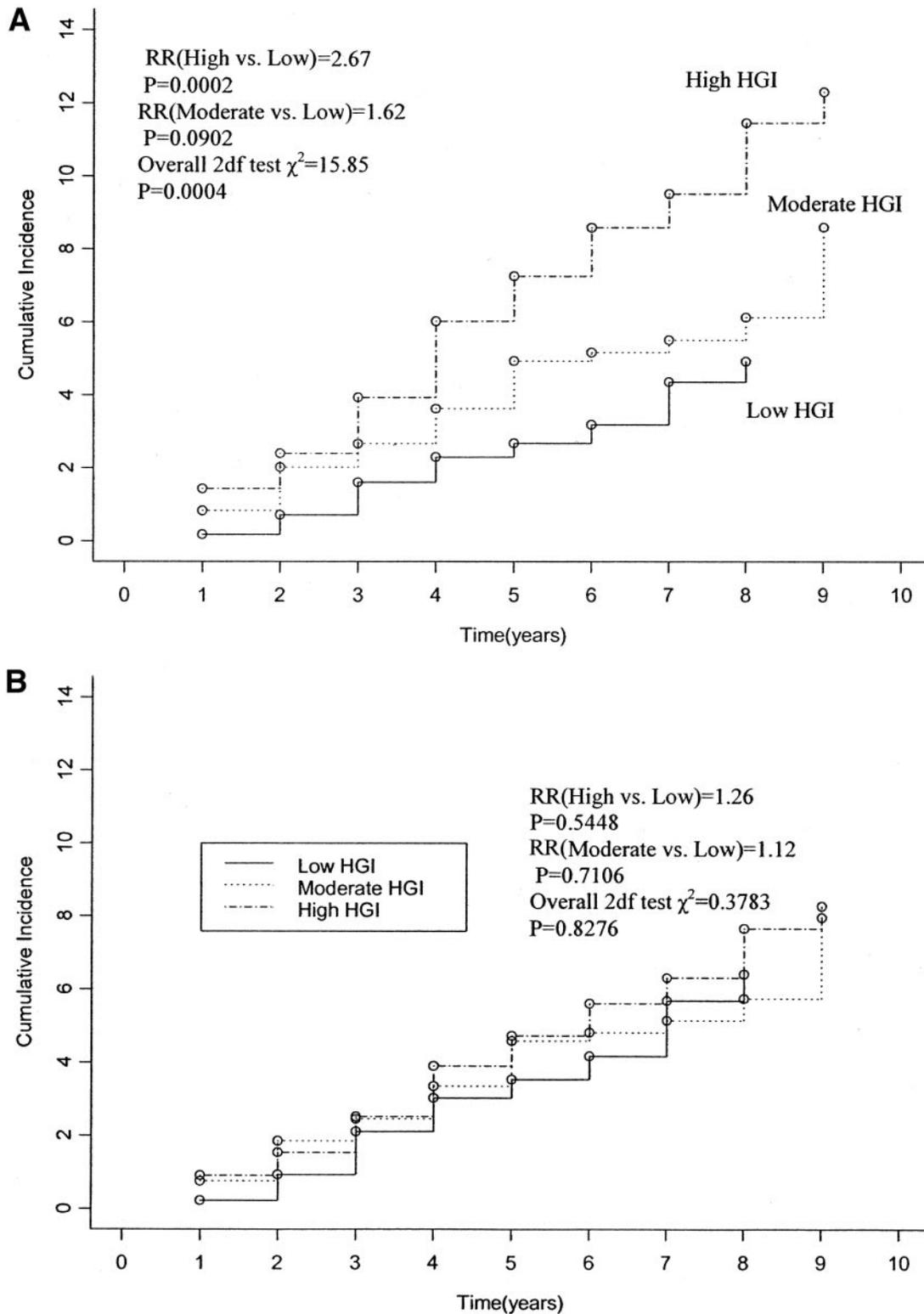
FIG. 4. Cumulative incidence of nephropathy progression (advanced microalbuminuria) within the high, moderate, and low HGI groups, obtained from a Cox proportional hazards regression model adjusted for the effects of age, diabetes duration, sex, treatment group, cohort, and MBG as a time-dependent covariate. The adjusted RR for moderate versus low HGI and for high versus low HGI with P value and the overall 2-df Wald test, χ^2, and P values are shown. A: Without adjustment for A1C. B: With adjustment for the A1C at eligibility and the time-dependent current updated mean A1C.

studies may have precluded having "high" or "low" glyca-tors in the study sample, the very high correlation coeffi-cients in the two independent studies suggest that variable glycation, if it exists, is not common.

To show the relationship between A1C and diurnal variation in blood glucose levels with precision, continu-ous monitoring of blood glucose levels with frequent measures of A1C over many months would be required. If long-term data related to progression of complications were also available, such data might provide a refined
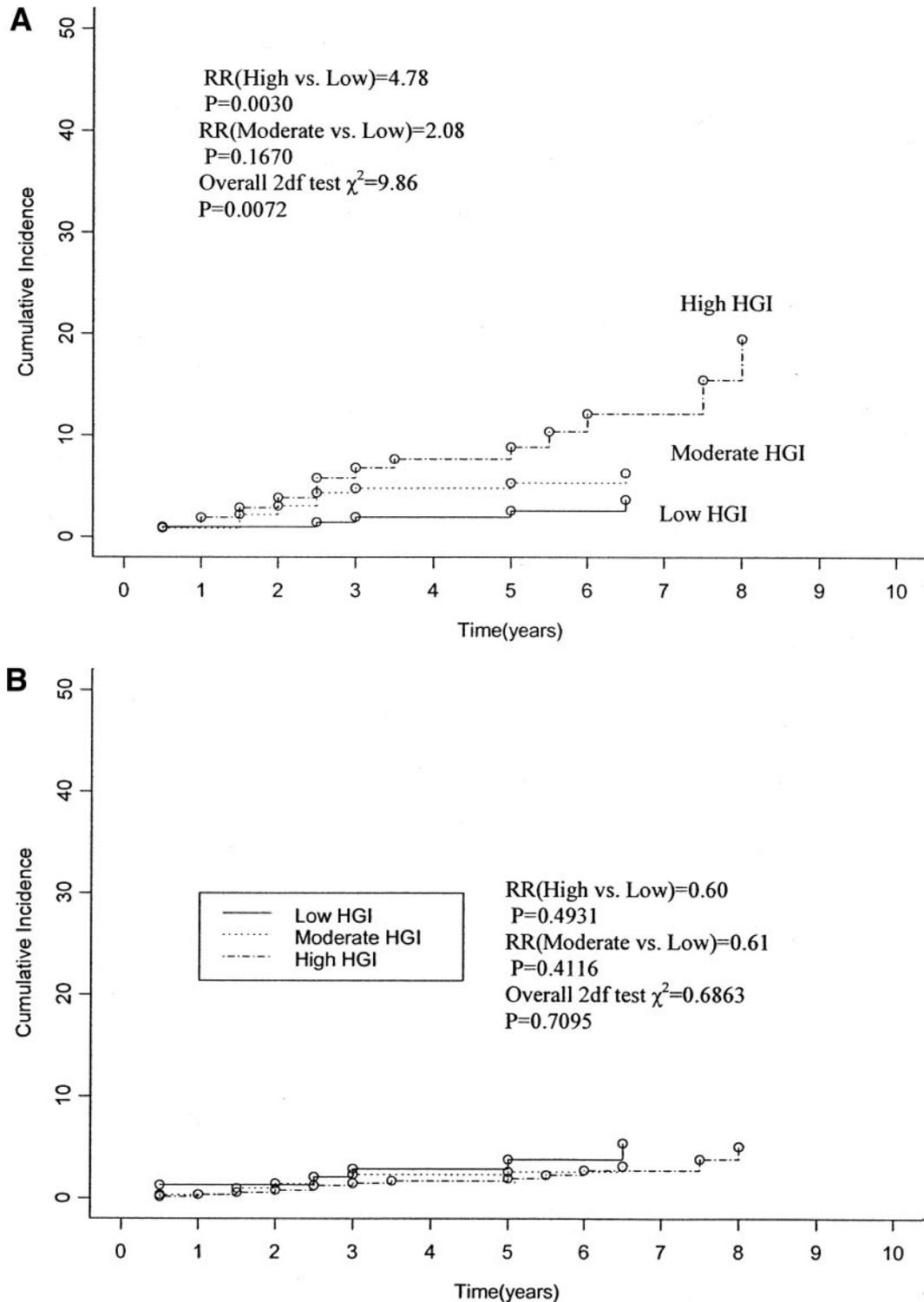
FIG. 5. Cumulative incidence of retinopathy progression within the high, moderate, and low HGI groups for those subjects within the low MBG group, obtained from a Cox proportional hazards regression model adjusted for the effects of age, diabetes duration, sex, treatment group, cohort, and MBG as a time-dependent covariate. The adjusted RR for moderate versus low HGI and for high versus low HGI with P value and the overall 2-df Wald test, χ^2, and P values are shown. A: Without adjustment for A1C. B: With adjustment for the A1C at eligibility and the time-dependent current updated mean A1C.

glycation index that might be shown to be an independent risk factor for progression of complications. However, from the data available in the DCCT restricted to quarterly contemporaneous levels of MBG and A1C, the calculated glycation index is not likely to add to the association with risk of complications after adjusting for the level of A1C.

Another factor that may contribute to biological variation in A1C is variation in the longevity of red cells (16). For a given history of 24-h blood glucose levels, variation between subjects in the red cell lifespan would be expected to result in variation in the A1C level and in the glycation index, which is by definition the deviation of the
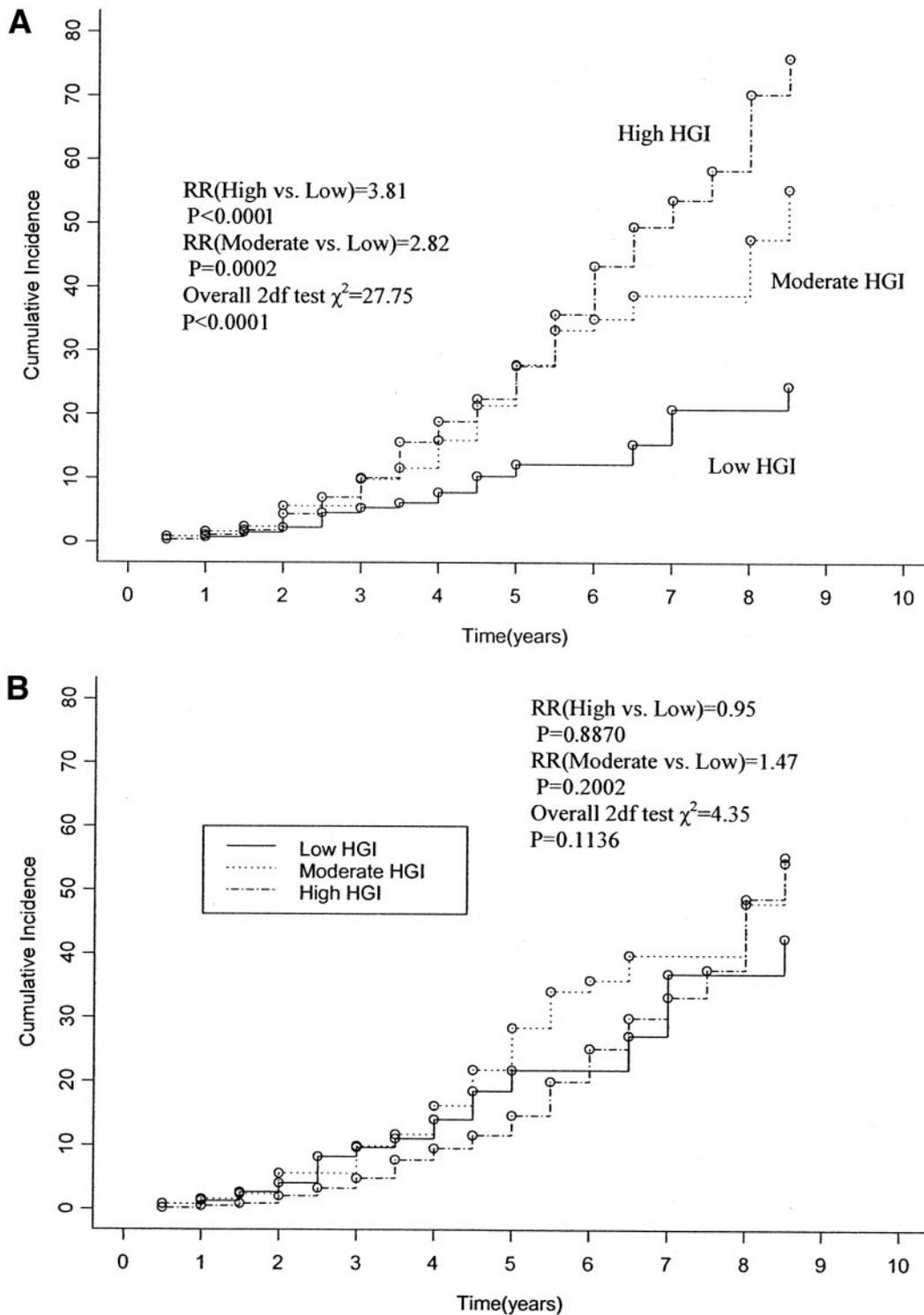
FIG. 6. Cumulative incidence of retinopathy progression within the high, moderate, and low HGI groups for those subjects within the high MBG group, obtained from a Cox proportional hazards regression model adjusted for the effects of age, diabetes duration, sex, treatment group, cohort, and MBG as a time-dependent covariate. The adjusted RR for moderate versus low HGI and for high versus low HGI with P value and the overall 2-df Wald test, χ^2, and P values are shown. A: Without adjustment for A1C. B: With adjustment for the A1C at eligibility and the time-dependent current updated mean A1C.

observed from expected level of A1C for a given glucose profile. An inverse correlation between erythrocyte survival and A1C level has recently been reported, suggesting that an adverse effect of poor glycemic control on erythrocyte lifespan may complicate this relationship (17).

Other evidence offered in support of inter-individual variation in glycation at equivalent blood glucose levels is the presence of a heritability factor that has been suggested to influence A1C levels (18). Although correlations of 0.68 between the A1C levels of twins concordant for type 1 diabetes and 0.52 between A1C levels of twins discordant for diabetes were reported, there was no

independent measurement of glucose levels in the diabetic twins. Thus, whether the degree of concordance in A1C levels was related to the concordance of average glycemia or to some other factor, such as genetically mediated rates of glycation, could not be determined.

There have been few analyses of the association of the levels of blood glucose with risk of complications. Based on analyses of a portion of the DCCT data, Service et al. (19) reported that the updated MBG is a risk factor for retinopathy. However, it is also important to note that adjustment for the MBG during follow-up in the DCCT did not significantly alter the association of the current mean A1C with risk of complications and was not an independent risk factor when added to A1C. Kilpatrick et al. (20) recently analyzed the DCCT data and showed that within-day and between-day variability in blood glucose levels from the quarterly Profilsets were not associated with risk of complications. Thus, all available analyses show that the strongest predictor of risk of complications is the A1C itself, not the level of blood glucose or the glycation index.

Formation of advanced glycation end products (AGEs) is thought to constitute one pathway in the pathogenesis of microvascular complications (21). There is evidence from a DCCT ancillary study that glycation of collagen, reflected in its furosine content, and further formation of AGEs, reflected in its carboxymethyllysine content, are predictive of the development and progression of diabetic retinopathy and nephropathy, independent of A1C levels (22,23). These analyses show that the levels of these AGEs may be a mechanism by which a sustained higher level of A1C results in a higher risk of progression of complications.

A variable propensity to glycate proteins other than hemoglobin, possibly influenced by genetic factors, could contribute to the risk of complications, over and above that attributable to chronic hyperglycemia. However, the analyses herein show that it is the level of A1C, not the purported HGI, that determines this risk.

In conclusion, our analyses show that subjects with different levels of the HGI also have different levels of A1C. Although higher values of the glycation index alone are associated with higher risk of complications, the effect on risk is wholly due to the correlation between the glycation index and the level of A1C. For subjects with the same level of A1C, differences in the glycation index have no effect on differences in risk of complications. Therefore, we reject the suggestion of McCarter et al. (4), based on DCCT HBA1c and blood glucose data, that subjects with a propensity to glycation have an increased risk of complications regardless of their A1C. Rather, our data show that subjects with similar lifetime exposures to glycemia (A1C) will have similar risks, and that the lower the lifetime levels of A1C, the lower the risks of complications, regardless of the level of the purported HGI.

Clinicians and investigators should not replace or supplement the A1C level with the HGI to estimate the risk of complications in individual patients or in groups of patients or to determine the need for intensification of therapy. Clinicians should strive to meet the recommended A1C and glucose targets in all patients and should disregard the level of the HGI in doing so.

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