Relationship of Prospective GHb to Glycated Serum Proteins in Incident Diabetic Retinopathy

Implications of the glycation gap for mechanism of risk prediction

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When estimating long-term glycemic control, A1C is considered the gold standard (1–3), but patients with seemingly equivalent A1C differ in their risk for microvascular complications (4,5). Recently, the "glycation gap," defined as the difference between the measured A1C and that which would be predicted from another measure of glycemic control, fructosamine, has been proposed as a means of identifying sources of variance in the apparent risk (6). Because hemoglobin is an intracellular protein and fructosamine reflects extracellular proteins, the glycation gap could result from differences between the ambient glucose concentrations or rates of glycation in the intracellular and extracellular compartments or interindividual differences in the turnover/metabolism of underlying proteins (6). In this study, we sought to determine whether there are differences in the relationship of GHb to fructosamine in diabetic subjects who do or do not develop retinopathy.

RESEARCH DESIGN AND METHODS

— The present study was completed in collaboration with the Wisconsin Diabetes Registry Study (WDRS), an incident type 1 diabetes cohort followed for complications over 4–14 years' duration. The WDRS has been described previously (7). New fructosamine testing was completed in 86 subjects who were identified among 290 with fundus photographs at 9 years. Patients with retinopathy ($n = 13$), patients with missing photographs indicating no retinopathy ($n = 38$) at 4 years or missing GHb or random glucose at 4 and/or 9 years ($n = 118$), and patients having insufficient plasma for testing fructosamine at the 4-year exam ($n = 35$) were excluded. Of the 86 eligible patients, 2 with fructosamine concentrations >1,000 μmol/l were omitted. Of the 84 patients included, 42 had retinopathy at 9 years.

Retinal status was assessed using a severity scale developed for the Wisconsin Epidemiologic Study of Diabetic Retinopathy (8,9). Total GHb was determined using microcolumn affinity chromatography within 7 days of sample collection (Isolab Glycaffin; Isolab, Akron, OH; intra-assay coefficient of variation [CV] 1.1%) (10). Fructosamine assays used a Roche kit Hitachi autoanalyzer; determinations were performed by single assay of samples stored at −80°C for intervals of 9–14 years (intra-assay CV 1.2%). The mean CV of a panel of seven control samples measured over a 35-month period was 4% (range 2–6).

The glycation gap was computed as previously described (6). A bootstrap technique was used to derive the parameter estimates that would be obtained from an independent reference sample drawn from the same population. The Nagelkerke R^2 was used to determine the relative contribution of predictor variables to the model (11).

RESULTS

— The groups did not differ in age, sex, race, or duration of diabetes (Table 1). GHb, fructosamine, and random glucose measured at the 4-year exam were significantly higher in those who subsequently developed retinopathy than in those who did not; the difference in GHb has been previously reported (7). Five subjects who developed retinopathy and two who did not met criteria for microalbuminuria at 4 years ($P = 0.23$), but mean urinary albumin excretion was normal in both groups (data not shown). There is a significant difference in the glycation gap between those with and without subsequent retinopathy; the mean glycation gap is positive in those with retinopathy but negative in those without. Stated differently, the two groups were not symmetrically distributed about the regression line of GHb on fructosamine (supplemental Fig. 1, available in an online appendix at http://dx.doi.org/10.2337/dc07-1465), as would be predicted if glycemic control were the sole mediator of risk prediction by either GHb or fructosamine. Altogether, 21 of the affected and 13 of the unaffected patients were above the line, while 21 of the affected and 29 of the unaffected patients were below the line (suppl. Fig. 1; χ^2 $P = 0.014$).

The R^2 for the regression of GHb on fructosamine was 0.33, suggesting that the glycation gap accounted for 67% of the variance in GHb (suppl. Fig. 1). Models predicting retinopathy were based on the notion that GHb is an independent predictor of retinopathy that should equate in predictive accuracy to a model containing both glycation gap and fruc-

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A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Table 1—*Mean ± SD or frequency (%) for pertinent 4-year exam data with significance of the differences and logistic regression analysis*

	No retinopathy (n = 42)	Retinopathy (n = 42)	OR (95% CI)	R^2	P
Age (years)	13.7 ± 7.8	15.3 ± 6.4			0.33
Male	17 (40.5)	25 (59.5)			0.126
Female	25 (59.5)	17 (40.5)			
Nonwhite	0 (0)	2 (4.8)			0.494
White	42 (100)	40 (95.2)			
UAE (mg/min)	6.5 ± 0.4	14.1 ± 25.2			0.037
Duration of diabetes (years)	3.4 ± 0.3	3.4 ± 0.3			0.625
Glucose (mg/dl)	195 ± 100	236 ± 101			0.061
GHb (%)	9.9 ± 2.1	12.1 ± 3.2	1.435 (1.150–1.791)	0.214	0.001
Fructosamine (μmol/l)	471 ± 137	565 ± 136	1.005 (1.002–1.009)	0.144	0.004
Glycation gap (%)	−0.6 ± 1.8	0.6 ± 2.8	1.252 (1.025–1.528)	0.083	0.028
Fructosamine +			1.006 (1.002–1.010)		0.002
Glycation gap			1.341 (1.055–1.703)	0.238	0.016

UAE, urinary albumin excretion.

tosamine (i.e., the two components of GHb). Indeed, the accuracy of predictions for GHb was indistinguishable from the model combining fructosamine and glycation gap (C-statistic [a measure of the accuracy of predictions] for GHb alone: 0.726 [95% CI 0.618–0.835]; C-statistic for fructosamine + glycation gap: 0.747 [0.643–0.851]). However, the ratio of the Nagelkerke R^2 for the model containing glycation gap only to that containing both fructosamine and glycation gap suggests that the glycation gap accounts for 39% of the ability of GHb to predict retinopathy (Table 1).

CONCLUSIONS — The probability of developing retinopathy increased with increasing GHb and with another measure of glycemic control, fructosamine. There was insufficient microalbuminuria at 4 years to account for any difference in serum fructosamine between the groups (12). Fructosamine contributed about three-fifths to the predictive power of GHb, while the glycation gap contributed about two-fifths, suggesting that in this sample a substantial portion of the ability of GHb to predict diabetic retinopathy is due to properties it does not share in common with an alternative measure of glycemic control.

This attribute of the glycation gap representing a fraction of the variance in GHb independent of glycemic control (6) and its heritability (13,14) support the notion that the ability of GHb to predict diabetes complications may not reside solely in its ability to reflect average glycemic control. Though it is possible that interindividual differences in protein turnover would contribute, it is more likely that either the glucose to which the proteins are exposed or the rate of glycation/deglycation (15) accounts for the difference in glycation gap between affected and unaffected individuals. Aside from intracellular concentration differences, largely determined by the facilitative glucose transporter GLUT1 (16), there could be factors, including genetic ones, inside the cell affecting the rate of either nonenzymatic glycation or enzymatic deglycation (17,18). Previous observations that GHb but not fructosamine is in part genetically determined (13) support this possibility.

It is important to stress that the glycation gap, similar to the "hemoglobin glycation index (HGI)" (19), is not independent of GHb (20,21); since the glycation gap is computed as the difference between a measured and a predicted A1C (or in this case GHb), independence is impossible. However, the glycation gap is not dependent on glycemic control, as indicated by the lack of correlation with fructosamine. Thus, the value of either the glycation gap (6) or the HGI (22) is that they represent a means of estimating the sources of variability in A1C that are shared in common with another measure of glycemic control and those that are unshared and therefore potentially due to other mechanisms (23,24). A1C is not synonymous with glycemic control, and it may be that some of the factors altering A1C apart from glycemic control also alter risk of diabetes complications, which is what is captured in the risk prediction of glycation gap.

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