Discordance Between HbA$_{1c}$ and Fructosamine

Evidence for a glycosylation gap and its relation to diabetic nephropathy

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OBJECTIVE — Discordances between HbA$_{1c}$ and other measures of glycemic control are common in clinical practice and remain unexplained. We developed a measure of discordance between HbA$_{1c}$ and fructosamine (FA) (glycosylated serum proteins) to conduct a systematic evaluation. We termed this the glycosylation gap (GG) and sought to determine its relationship to diabetic nephropathy.

RESEARCH DESIGN AND METHODS — Measurements of HbA$_{1c}$ and FA on the same sample in 153 people were used to calculate GG, defined as the difference between measured HbA$_{1c}$ and HbA$_{1c}$ predicted from FA based on the population regression of HbA$_{1c}$ on FA.

RESULTS — GG had a broad distribution (range, -3.2% to 5.5%); 40% of samples had values indicating major differences in prediction of complications risk by the measured versus predicted HbA$_{1c}$. GG was highly correlated ($r = 0.81$) between measurements repeated in 65 patients 23 ± 2 weeks apart, indicating that the discordances are reliable and not explained by differences in turnover of underlying proteins. In 40 patients with type 1 diabetes of ≥ 15 years' duration, an increase in GG by 1% was associated with a 2.9-fold greater frequency of increasing nephropathy stage ($P = 0.0014$). GG was $-0.8 \pm 0.2\%$ in subjects with no nephropathy, $-0.3 \pm 0.2\%$ with microalbuminuria/hypertension, and $0.7 \pm 0.3\%$ in subjects with proteinuria or renal dysfunction ($P < 0.05$). GG correlated better with nephropathy than did either HbA$_{1c}$ or FA alone in this population.

CONCLUSIONS — The glycosylation gap may be a useful clinical research tool for evaluating physiologic sources of variation in diabetic complications beyond glycemic control.

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H bA$_{1c}$ is regarded as the gold standard for measurement of glycemic control and is invaluable in the treatment of diabetic patients. It is a predictor of diabetic complications, and interventions that reduce HbA$_{1c}$ correspondingly reduce the risk of complications (1–4). Whereas the overall utility of HbA$_{1c}$ is beyond question, discordances between HbA$_{1c}$ and other measures of glycemic control are commonly encountered (5–7) and are important because of the millions of yearly HbA$_{1c}$ measurements. Discordances between HbA$_{1c}$ and other clinically used biochemical measures of glycemic control have often been attributed to inadequacies or poor reliability of glucose self-testing and reporting or artifacts (5–8). Despite newer, more reliable technology, however, significant differences between measures of plasma glucose and glycemia estimated from HbA$_{1c}$ are still encountered. The 6- to 12-week time frame over which HbA$_{1c}$ equilibrates is an important consideration when comparing with shorter-term measures of plasma glucose, but temporal factors may not entirely account for persistent discordance (9–15).

The question arises whether discordances between HbA$_{1c}$ and other measures of glycemia are due to physiologic processes consistent within individuals over time. If this is the case, it is plausible that variation among diabetic patients in the intracellular glycation of proteins, independent of plasma glycemia, could affect the frequency of diabetic complications. We therefore wanted to test whether the discordance between HbA$_{1c}$ and a simple measure of integrated plasma glucose was reliable and bore a relationship to the frequency of a major complication, diabetic nephropathy. An important difference between plasma glucose and HbA$_{1c}$ is that the former reflects the physiology of glucose in the extracellular space, whereas HbA$_{1c}$ reflects nonenzymatic glycosylation (and depends on glucose concentration) in the intra-erythrocyte compartment. We therefore selected fructosamine (FA) for comparison because it represents a clinically accessible measure of nonenzymatic glycation of proteins in the same compartment as plasma glucose, and should integrate plasma glucose fluctuations. The common perception is that FA results differ from HbA$_{1c}$ exclusively because of the difference in turnover times of the underlying glycation targets, hemoglobin versus serum proteins. The difference in physiologic compartments reflected in FA and HbA$_{1c}$ has not been the object of interest. It was therefore important to exclude the effect of the difference in turnover time by evaluating the reliability of discordances over a greater time than that in which the turnover of red cells or most serum proteins occurs. The study was conducted with prospective collec-

tion of HbA$_{1c}$ and FA in the population, with assessment of diabetic nephropathy based on chart review.

RESEARCH DESIGN AND METHODS

HbA$_{1c}$-FA regression
Patient population. HbA$_{1c}$ and FA were both measured on the same sample from 153 people seen for diabetes drawn randomly from a university hospital–based diabetes clinic and private endocrine office practice. Exclusions included known hemoglobinopathies or erythrocyte disorders.

Mathematical methods. The glycosylation gap (GG) was calculated as the difference between measured HbA$_{1c}$ and HbA$_{1c}$ predicted from the FA based on the HbA$_{1c}$-FA regression equation

$$GG = \text{measured HbA}_{1c} - \text{predicted HbA}_{1c}$$

By this definition, GG is negative if measured HbA$_{1c}$ is less than HbA$_{1c}$ predicted from FA and positive if measured HbA$_{1c}$ is greater than predicted. GG is zero when HbA$_{1c}$ and FA are concordant. For example, HbA$_{1c}$ at the lower limit of normal and FA above the upper limit of normal would result in a negative GG, meaning the HbA$_{1c}$ is lower than predicted from the FA. If HbA$_{1c}$ is disproportionately higher than FA in patients with a complication of diabetes, increasing complications would be predicted as the gap becomes more positive, and conversely. No association would be expected if GG is random.

In subjects with repeated measurements of HbA$_{1c}$ and FA, the first and second pairs of values were used to examine variation in GG with time. If GG represented random variation in the HbA$_{1c}$-FA regression or resulted from differing time windows of exposure of serum albumin and the erythrocyte to glucose, consistency would not be expected.

Data were analyzed using JMP statistical software (SAS Institute, Cary, NC).

Nephropathy staging
The analysis of nephropathy was performed in a subset population with type 1 diabetes of $\geq$15 years' duration according to the following criteria: Grade 0, normal renal function: urinary microalbumin $\leq$20 μg/min (24-h urine) or $\leq$17.9 μg/mg creatinine (spot urine); diastolic
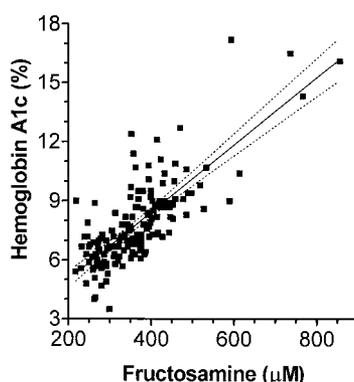
Figure 1—*HbA$_{1c}$ and FA measured on the same sample in 153 patients. The regression line (solid) for the whole sample is HbA$_{1c}$ = 0.017 $\times$ FA + 1.61 (r = 0.78), with 95% confidence limits for the regression (dotted lines). Demographics: age, 47 $\pm$ 17 years; gender, 84 (55%) women and 69 (45%) men; race/ethnicity, 120 (78%) Caucasian, 28 (18%) African-American, 3 (2%) Asian-American, 2 (1%) unknown; diagnosis, 72 (47%) type 1 diabetes, 71 (46%) type 2 diabetes, 10 (7%) diabetes, type unknown.*

blood pressure <95 mmHg without antihypertensive therapy.

Grade 1, microalbuminuria or hypertension: urine microalbumin 20–200 μg/min (24 h) or 17.9–179 μg/mg creatinine (spot); or diastolic blood pressure >95 mmHg; or treatment for hypertension.

Grade 2, advanced renal dysfunction: >200 μg/min urinary microalbumin or >300 mg/24 h protein or serum creatinine >1.5 mg/dl.

Blood pressure was measured using a mercury sphygmomanometer with cuff size selected based on manufacturer's recommendation for arm diameter with the subject seated for 5 min.

Analytic methods
HbA$_{1c}$, FA, urinary albumin, protein, and/or creatinine, and serum creatinine were measured during routine clinical care without batching. HbA$_{1c}$ was measured in the laboratory of the Health Alliance of Greater Cincinnati using high-performance liquid chromatography (HPLC) ion exchange (Tosoh, Tokyo, Japan) (interassay coefficient of variation 2.9% at HbA$_{1c}$ 5.5%, 1.9% at HbA$_{1c}$ 10.0%). According to the manufacturer, carbamylated Hb does not interfere with the determination of HbA$_{1c}$ in this assay. Fructosamine was measured at Quest Diagnostics by autoanalyzer (Roche Diagnostics, Indianapolis, IN) with a nitroblue

tetrazolium reaction, using a fructose-polylysine standard, interassay coefficient of variation 2%. Urinary microalbumin was measured by nephelometry, and urine protein was measured spectrophotometrically.

RESULTS
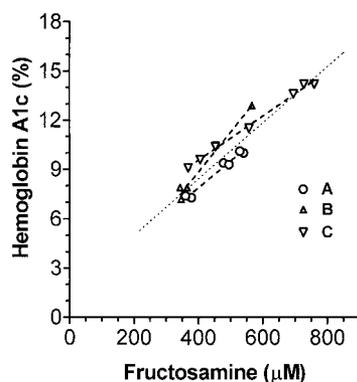
HbA$_{1c}$-FA regression
HbA$_{1c}$ and FA were highly correlated (r^2 = 0.61) (Fig. 1) in a population with widely varying glycemic control (see demographics in Fig. 1 legend): HbA$_{1c}$ (mean $\pm$SD) was 7.9 $\pm$ 2.2%, FA 367 $\pm$ 98 μmol/l. The degree of correlation is very similar to previous reports (7–12,14,16). The regression line did not differ whether determined on the whole population or with exclusion of patients with type 1 diabetes of >15 years' duration, who form the basis for later analyses. Although there is a high degree of correlation, it is evident that there is substantial scatter as well. There was no difference in HbA$_{1c}$ and FA or the regression line between HbA$_{1c}$ and FA or GG by sex.

Reliability and population variation of the glycosylation gap
To assess reproducibility of the glycosylation gap, we analyzed the relationship between consecutive determinations in 65 pairs of HbA$_{1c}$ and FA collected 23 $\pm$ 2 weeks apart (Fig. 2). There was a strong correlation (r = 0.81) between the first and second determinations, with a y-intercept that did not differ from 0 and slope that was positive but not equal to 1. The paired measurements did not differ (t test). Based on that, the sign of GG ap-
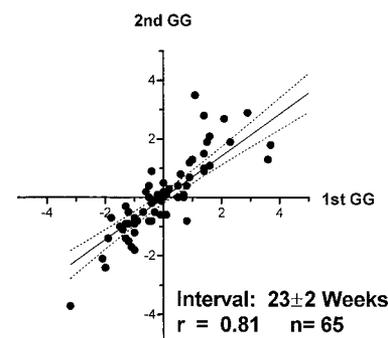
Figure 2—*Relationship between paired glycosylation gaps determined within a subject on two separate occasions. Measurements were performed at follow-up visits with the indicated average interval.*

Figure 3—*Regression lines for serial samples of paired HbA$_{1c}$ and FA in individual subjects A–C plotted in relationship to the regression line for the whole population from Fig. 1.*

pears highly reproducible, and the magnitudes of repeated determinations closely related. This is demonstrated over a time frame long enough to exclude effects of the physiologic differences in albumin versus erythrocyte turnover on GG.

To better assess intraindividual variability in the relationship of HbA$_{1c}$ to FA, the regression was plotted for individual subjects in whom multiple pairs of HbA$_{1c}$ and FA data over a time series were available (Fig. 3). The paired values could be highly correlated ($r \geq 0.98$ in each case shown) suggesting a fundamental physiologic relationship. The line of one subject (A) appears parallel to the population line, indicating that this individual's GG would be constant across the range of glycemic control. One example each is shown of subjects in whom the slope of the line is higher (B) or lower (C) than that for the population. These predict average GGs within subjects that are positive in both cases but rising in one case and falling in the other case as glycemic control deteriorates. This is a potential factor in the variability in magnitude of GG while the sign is more reproducible.

The distribution of GG was determined in the original population from which the measure was derived and in the subpopulation with type 1 diabetes of ≥15 years' duration. The distribution of GG in the original population was skewed toward positive values and not normally distributed (Shapiro-Wilk test), with median −0.20% and 25th and 75th percentiles of −0.8% and 0.7% (not shown). In the long-term type 1 diabetes population, the distribution of GG was normally dis-

tributed with mean ± SD −0.3% ± 1.0% (not shown). Twenty-seven (17%) and 36 (23%) patients had GG >1% below or above zero, respectively, indicating a large population in whom prediction of complications on the basis of measured HbA$_{1c}$ would differ substantially from that based on HbA$_{1c}$ predicted from FA and the HbA$_{1c}$-FA regression. An HbA$_{1c}$ decrement of 1–2% has reduced microvascular complications 40–70% (2–4).

The glycosylation gap and nephropathy stage
Among subjects with type 1 diabetes of ≥15 years' duration, the glycosylation gap was −0.7 ± 0.2% with no nephropathy, −0.3 ± 0.2% with microalbuminuria/hypertension, and 0.7 ± 0.3% with proteinuria or severe renal dysfunction (Fig. 4) ($P = 0.005$ by ANOVA; $P < 0.05$ for a difference between stage 0 and stage 2 by post-hoc Student's t test). By ordinal logistic regression, there was a highly significant relationship found between GG and nephropathy: the relative frequency of advancing nephropathy stage, normalized per HbA$_{1c}$ unit, for GG was 2.9-fold (95% CI 1.4–6.1; $P = 0.003$); for HbA$_{1c}$, 1.3 (0.86–1.9; $P = 0.21$); and for FA 0.84 (0.59–1.2; $P = 0.31$). Of the 10 patients described as stage 2, a single patient was in chronic renal failure (not on dialysis) and one other patient had had a renal transplant. Relationships between nephropathy stage and either HbA$_{1c}$ or FA did not reach significance. After correction for the effects of urinary protein loss, the relative frequency of advancing nephropathy stage, normalized per HbA$_{1c}$ unit, for GG was 2.2-fold (95% CI 1.1–4.5; $P = 0.03$). Mean ± SE FA was 387 ± 11 μmol/l for all the subjects with type 1 diabetes of ≥15 years' duration, 394 ± 17 μmol/l in subjects with no nephropathy, 378 ± 21 μmol/l in those with microalbuminuria, and 386 ± 23 μmol/l in those with proteinuria, indicating no significant decline with worsening nephropathy ($P = 0.82$). HbA$_{1c}$ was 7.9 ± 0.2% in the entire subpopulation with diabetes of ≥15 years' duration, 7.6 ± 0.4% in subjects with no nephropathy, 7.7 ± 0.4% with microalbuminuria, and 8.7 ± 0.5% with proteinuria ($P = 0.15$). The regression between GG and nephropathy was not altered when HbA$_{1c}$, FA, or duration of diabetes were included as factors in the analysis. Results were similar when GG was expressed as a percentage of measured

HbA$_{1c}$, which would normalize for HbA$_{1c}$ absolute magnitude. There was no effect of sex detected on multivariate ordinal logistic regression between glycosylation gap and nephropathy.

If the glycosylation gap were substantially altered by the presence of diabetic nephropathy, the population with long-term diabetes, which includes those with nephropathy, might be expected to have higher glycosylation gaps than those with short-duration type 1 diabetes. However, no difference was detected in the glycosylation gap between the subjects with type 1 diabetes of long versus short duration (−0.4 ± 0.2% vs. −0.5 ± 0.1%; $P = 0.63$, Wilcoxon test).

We sought to model the effects of glycated proteinuria on glycosylation gap. There is evidence that glycated albumin is no more likely to be lost in the urine than nonglycated albumin (17–19). At total body albumin daily synthesis of 13.9 g/person reported in patients with type 1 diabetes and nephropathy (20), GG would be altered by 0.1% for each 213 mg/24 h of urinary albumin at the group mean FA of 386. Albumin constitutes ~85% of the total urinary protein in diabetic nephropathy (21). When these factors were taken into account, the relative frequency of advancing nephropathy stage with GG remained significant, 2.2-fold per HbA$_{1c}$ unit (95% CI 1.1–4.5; $P = 0.03$).

CONCLUSIONS
This study demonstrates that discordances commonly found among measures of glycemic control can be persistent and stable and related to a major complication of diabetes, nephropathy. The fact that the glycosylation gap, the HbA$_{1c}$-FA residual, correlates so well with a major complication of diabetes suggests shared factors between the origin of the discordance and the pathogenesis of the disease process. What does it mean that this study did not detect a relationship between HbA$_{1c}$ and nephropathy? The population was relatively small and the study may not have had the power to detect that effect.

The glycosylation gap could be affected by the production and disappearance rate of glycated hemoglobin, glycated serum proteins, or both. We are particularly interested in analyzing the variations found in otherwise healthy people with diabetes in whom urinary protein loss is not an issue, specifically
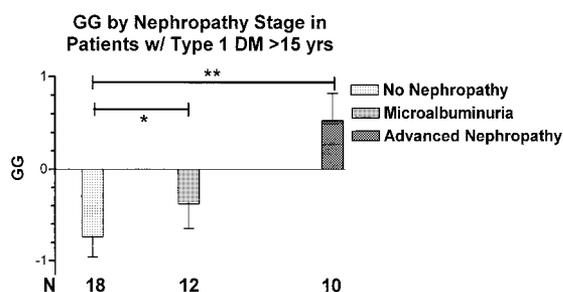
Figure 4—*Mean GG in patients with type 1 diabetes of ≥15 years' duration as a function of nephropathy stage (as defined in RESEARCH DESIGN AND METHODS). Demographics: age, 44 ± 11 years; diabetes duration, 30 ± 10 years; gender 18 (45%) women and 22 (55%) men; race/ethnicity, 38 (95%) Caucasian, 2 (5%) African-American. For presence of a difference among the groups by ANOVA, P = 0.005. For differences between specific groups by post-hoc t test: * No significant difference; **P < 0.05.*

whether it results predominantly from a gradient in protein nonenzymatic glycosylation between the two physiologic compartments. It is, however, possible that variations in the GG could be a consequence, rather than a marker or predictor, of nephropathy due to effects of proteinuria on protein turnover and FA. Patients with a high GG and high risk of nephropathy have a low FA in relationship to the HbA$_{1c}$. This would be the direction anticipated if albumin has a shorter survival time in the circulation, upon loss of glomerular selectivity (18,19). However, the lack of significant difference in FA by nephropathy stage is itself an argument that the findings are not explained by reduced FA in the group with urinary protein loss. Urine protein loss can contribute to the GG and can have a quantitatively important effect when present in the high microalbuminuric range. However, our modeling shows that a rise in glycosylation gap remained associated with increased nephropathy frequency even when such effects were taken into account. The observed effects are not solely a consequence of urinary protein loss. We were unable to detect a difference in GG by duration of type 1 diabetes expected if the gap is a consequence of nephropathy. Markedly positive GG values were found in patients with shorter durations of diabetes than those included in the complications analysis who do not currently have significant nephropathy, indicating that nephropathy is not necessary to have a positive GG. Other unrelated disease states that markedly perturb Hb or serum protein turnover could affect the GG, so these measurements must be assessed in clinical context.

These data demonstrate correlation between GG and nephropathy, not prediction. Hempe et al. (22) and McCarter et al. (23) have demonstrated that the hemoglobin glycosylation index, an analogous measure of discordance between self glucose–monitoring and HbA$_{1c}$, is also consistent and predicts both retinopathy and nephropathy, suggesting that the differences between extracellular and intracellular measures of glycemic control may precede nephropathy. Future prospective studies could delineate the cause-and-effect relationship in the regression of GG on nephropathy stage. The parallel between the Hempe et al. studies and ours that the discordances are between HbA$_{1c}$ and extra-erythrocyte measures of glycemic control strongly favors the concept that the discordances are systematic and potentially relevant to the pathophysiology of diabetic complications. It would also argue for an underlying mechanism that is related to the difference between the erythrocyte and plasma compartments. If, under carefully selected circumstances, the GG results predominantly from a gradient in protein nonenzymatic glycosylation between the plasma and intracellular compartments, it could represent previously unrecognized gradients in glucose concentration or factors that differentially alter the forward or reverse rates of nonenzymatic glycosylation. Variation in oxidation-reduction status (24,25) would be one important candidate. We have recently demonstrated population heterogeneity in the gradient established across the erythrocyte membrane by a radiolabeled nonmetabolizable glucose analog, 3-O-methylglucose, incubated with glucose-depleted erythrocytes (26). This supports the con-

cept of variability in the red cell membrane glucose gradient. The data do not distinguish among these or other candidate mechanisms.

Several caveats: The population used for the complications analysis was a subgroup of the population in which GG was defined rather than an independent population. However, the regression generated with a subpopulation that did not overlap with that for the complications analysis was identical. The nephropathy end points were based on American Diabetes Association standard of care Clinical Laboratory Improvement Amendments–certified routine clinical monitoring of patients, not research assays. Carbamylated hemoglobin can accumulate in erythrocytes of uremic patients (27–31), and it is important to ensure that carbamylated Hb is not a confounder. Whereas the manufacturer indicates that carbamylated Hb does not affect the method used here, there is limited evidence challenging this assertion (32). The urea nitrogen concentration was closely clustered in 38 of our 40 nephropathy analysis subjects, making a carbamylated Hb effect on these results unlikely (not shown). Most of the patients were at stable glycemic control when sampled, and Fig. 3 indicates that the HbA$_{1c}$-FA relationship can be extremely stable within subjects. If samples are collected early after changes in glycemic control, that could reduce the GG stability over time.

Shortened red cell survival associated with renal failure would have an effect opposite to the relationship found, making it unlikely to be a contributor to our findings.

In summary, we observed consistent discordances between HbA$_{1c}$ and an extra-erythrocyte measure of glycemic control, FA. Changes in glycosylated proteins resulting from nephropathy do not appear to explain these findings but have not been ruled out conclusively. When this discordance is quantitated in the form of a glycosylation gap, it correlates with the frequency of a major microvascular complication of diabetes. We propose that the glycosylation gap will be useful as a research probe quantitating variation between the two underlying tests of glycemic control to identify sources of population variation in diabetic complications beyond glycemic control per se. Prospective studies will be necessary to determine whether repeated determinations should

alter decision-making regarding risk for future complications in the individual patient.

References

1. Koenig RJ, Peterson CM, Jones RL, Saudek C, Lehrman M, Cerami A: Correlation of glucose regulation and hemoglobin AIc in diabetes mellitus. *N Engl J Med* 295:417–420, 1976
2. The Diabetes Control and Complications Trial Research Group: The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *N Engl J Med* 329:977–986, 1993
3. Ohkubo Y, Kishikawa H, Araki E, Miyata T, Isami S, Motoyoshi S, Kojima Y, Furuyoshi N, Shichiri M: Intensive insulin therapy prevents the progression of diabetic microvascular complications in Japanese patients with non-insulin-dependent diabetes mellitus: a randomized prospective 6-year study. *Diabetes Res Clin Pract* 28:103–117, 1995
4. UK Prospective Diabetes Study Group: Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). *Lancet* 352:837–853, 1998
5. Nathan DM, Singer DE, Hurxthal K, Goodson JD: The clinical information value of the glycosylated hemoglobin assay. *N Engl J Med* 310:341–346, 1984
6. Gonen B, Rubenstein A, Rochman H, Tanega SP, Horwitz DL: Haemoglobin A1: an indicator of the metabolic control of diabetic patients. *Lancet* 2:734–737, 1977
7. Kilpatrick ES: Problems in the assessment of glycaemic control in diabetes mellitus. *Diabet Med* 14:819–831, 1997
8. Cohen MP: *Diabetes and Protein Glycosylation: Measurement and Biologic Relevance.* New York, Springer-Verlag, 1986
9. Narbonne H, Renacco E, Pradel V, Portugal H, Vialettes B: Can fructosamine be a surrogate for HbA(1c) in evaluating the achievement of therapeutic goals in diabetes? *Diabete Metab* 27:598–603, 2001
10. Braatvedt GD, Drury PL, Cundy T: Assessing glycaemic control in diabetes: relationships between fructosamine and HbA1C. *NZ Med J* 110:459–462, 1997
11. Winocour PH, Bhatnagar D, Kalsi P, Hillier VF, Anderson DC: An analysis of glycosylated blood proteins and blood glucose profiles over one year in patients with type 1 diabetes. *Diabet Med* 6:709–716, 1989
12. Hom FG, Ettinger B, Lin MJ: Comparison of serum fructosamine vs glycohemoglobin as measures of glycemic control in a large diabetic population. *Acta Diabetol* 35:48–51, 1998
13. Tahara Y, Shima K: Kinetics of HbA1c, glycated albumin, and fructosamine and analysis of their weight functions against preceding plasma glucose level. *Diabetes Care* 18:440–447, 1995
14. Shield JP, Poyser K, Hunt L, Pennock CA: Fructosamine and glycated haemoglobin in the assessment of long term glycaemic control in diabetes. *Arch Dis Child* 71:443–445, 1994
15. Malik M, Gill GV, Pugh RN, Bakir A, Hossain M: Can plasma fructosamine substitute for glycated haemoglobin (HbA1c) estimation in the assessment of diabetic control? *Trop Doct* 30:74–76, 2000
16. Trenti T, Cristani A, Cioni G, Pentore R, Mussini C, Ventura E: Fructosamine and glycated hemoglobin as indices of glycemic control in patients with liver cirrhosis. *Ric Clin Lab* 20:261–267, 1990
17. Ishibashi F: Microalbuminuria in NIDDM is caused by increased excretion of unmodified albumin. *Diabetes* 45:731–735, 1996
18. Bent-Hansen L, Feldt-Rasmussen B, Kverneland A, Deckert T: Plasma disappearance of glycated and non-glycated albumin in type 1 (insulin-dependent) diabetes mellitus: evidence for charge dependent alterations of the plasma to lymph pathway. *Diabetologia* 36:361–363, 1993
19. Cavallo-Perin P, Chiambretti A, Calefato V, Tomalino M, Cecchini G, Gruden G, Pagano G: Urinary excretion of glycated albumin in insulin-dependent diabetic patients with micro- and macroalbuminuria. *Clin Nephrol* 38:9–13, 1992
20. Parving HH, Rossing N, Sander E: Increased metabolic turnover rate and transcapillary escape rate of albumin in long-term juvenile diabetics. *Scand J Clin Lab Invest* 35:59–66, 1975
21. Carrie BJ, Myers BD: Proteinuria and functional characteristics of the glomerular barrier in diabetic nephropathy. *Kidney Int* 17:669–676, 1980
22. Hempe J, Gomez R, McCarter R, Chalew SA: High and low hemoglobin glycation phenotypes in type 1 diabetes: a challenge for interpretation of glycemic control. *J Diabetes Complications* 16:313–320, 2002
23. McCarter R, Hempe J, Gomez R, Chalew SA: Hemoglobin glycosylation index is a predictor of complications in the DCCT (Abstract). *Diabetes* 49 (Suppl. 1):A48, 2000
24. Shinohara M, Thornalley PJ, Giardino I, Beisswenger P, Thorpe SR, Onorato J, Brownlee M: Overexpression of glyoxalase-I in bovine endothelial cells inhibits intracellular advanced glycation endproduct formation and prevents hyperglycemia-induced increases in macromolecular endocytosis. *J Clin Invest* 101:1142–1147, 1998
25. Wells-Knecht MC, Thorpe SR, Baynes JW: Pathways of formation of glycoxidation products during glycation of collagen. *Biochemistry* 34:15134–15141, 1995
26. Khera PK, Joiner CH, Holmes YR, Cohen RM: Evidence for a steady state glucose gradient across the erythrocyte membrane at 37 C: a potential source of variation in hemoglobin A1c (Abstract). *Diabetes* 50 (Suppl. 2):A176, 2001
27. Wynckel A, Randoux C, Millart H, Desroches C, Gillery P, Canivet E, Chanard J: Kinetics of carbamylated haemoglobin in acute renal failure. *Nephrol Dial Transplant* 15:1183–1188, 2000
28. Frazao JM, Barth RH, Berlyne GM: Carbamylated hemoglobin in prerenal azotemia. *Nephron* 71:153–155, 1995
29. Kwan JT, Carr EC, Bending MR, Barron JL: Determination of carbamylated hemoglobin by high-performance liquid chromatography. *Clin Chem* 36:607–610, 1990
30. Bry L, Chen PC, Sacks DB: Effects of hemoglobin variants and chemically modified derivatives on assays for glycohemoglobin. *Clin Chem* 47:153–163, 2001
31. Vaden SL, Gookin J, Trogdon M, Langston CE, Levine J, Cowgill LD: Use of carbamylated hemoglobin concentration to differentiate acute from chronic renal failure in dogs. *Am J Vet Res* 58:1193–1196, 1997
32. Weykamp CW, Miedema K, de Haan T, Doleman C: Carbamylated hemoglobin interference in glycohemoglobin assays (Letter). *Clin Chem* 45:438–440, 1999