

HbA$_{1c}$ Variability as an Independent Correlate of Nephropathy, but Not Retinopathy, in Patients With Type 2 Diabetes

The Renal Insufficiency And Cardiovascular Events (RIACE) Italian Multicenter Study

**OBJECTIVE**—To examine the association of hemoglobin (Hb) A$_{1c}$ variability with microvascular complications in the large cohort of subjects with type 2 diabetes from the Renal Insufficiency And Cardiovascular Events (RIACE) Italian Multicenter Study.

**RESEARCH DESIGN AND METHODS**—Serial (3–5) HbA$_{1c}$ values collected in a 2-year period before enrollment were available from 8,260 subjects from 9 centers (of 15,773 patients from 19 centers). HbA$_{1c}$ variability was measured as the intraindividual SD of 4.52 ± 0.76 values. Diabetic retinopathy (DR) was assessed by dilated funduscopy. Chronic kidney disease (CKD) was defined based on albuminuria, as measured by immunonephelometry or immunoturbidimetry, and estimated glomerular filtration rate (eGFR) was calculated from serum creatinine.

**RESULTS**—Median and interquartile range of average HbA$_{1c}$ (HbA$_{1c}$-MEAN) and HbA$_{1c}$-SD were 7.57% (6.86–8.38) and 0.46% (0.29–0.74), respectively. The highest prevalence of microalbuminuria, macroalbuminuria, reduced eGFR, albuminuric CKD phenotypes, and advanced DR was observed when both HbA$_{1c}$ parameters were above the median and the lowest when both were below the median. Logistic regression analyses showed that HbA$_{1c}$-SD adds to HbA$_{1c}$-MEAN as an independent correlate of microalbuminuria and stages 1–2 CKD and is an independent predictor of macroalbuminuria, reduced eGFR, and stages 3–5 albuminuric CKD, whereas HbA$_{1c}$-MEAN is not. The opposite was found for DR, whereas neither HbA$_{1c}$-MEAN nor HbA$_{1c}$-SD affected nonalbuminuric CKD.

**CONCLUSIONS**—In patients with type 2 diabetes, HbA$_{1c}$ variability affects (albuminuric) CKD more than average HbA$_{1c}$, whereas only the latter parameter affects DR, thus suggesting a variable effect of these measures on microvascular complications.

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Compelling evidence shows that long-term glycemic control, as expressed by hemoglobin (Hb) A$_{1c}$ levels, is the main risk factor for the development of microvascular complications in type 1 (1) and type 2 diabetes (2), with risk rising exponentially as HbA$_{1c}$ increases. Another risk factor related to hyperglycemia is variability of glycemic control that comprises “glucose variability” and “HbA$_{1c}$ variability.” Glucose variability relates to within-day fluctuations of glycemia, especially as a consequence of meals (3), and may eventually reflect in increased HbA$_{1c}$ levels. Conversely, HbA$_{1c}$ variability relates to changes in glycemia over longer periods of time that result in change in HbA$_{1c}$ from one visit to the next (4).

Retrospective analyses of data from the Diabetes Control and Complications Trial (DCCT) have not confirmed that within-day glucose variability predicts the development of microvascular complications (5–7), although this was not a prespecified end point of the study. However, a prospective study specifically addressing this issue did not show any effect of within-day glucose fluctuations on cardiovascular events (8). Conversely, retrospective analyses of the DCCT (9) and the Finnish Diabetic Nephropathy (FinnDiane) Study (10) have suggested that HbA$_{1c}$ variability is an independent risk factor.
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risk factor for the development of diabetic retinopathy (DR) and nephropathy (DN) in individuals with type 1 diabetes. Moreover, HbA1c variability was shown to be an independent variable that added to the effect of HbA1c on the risk of microalbuminuria in adolescent patients with type 1 diabetes from the Oxford Regional Prospective Study and the Nephropathy Family Study (11). Very recently, two prospective cohort studies from Japan and Taiwan, the Tsukuba Kawai Diabetes Registry 2 (12) and the Diabetes Management through an Integrated Delivery System project (13), have shown that HbA1c variability is associated with microalbuminuria, even after adjustment for known predictors of albuminuria, in 812 and 821 patients with type 2 diabetes, over a 4.3-year and a 6.2-year follow-up, respectively.

To further address this issue, we used the large cohort of Caucasian subjects with type 2 diabetes from the Renal Insufficiency And Cardiovascular Events (RIACE) Italian Multicenter Study to assess whether the baseline status of DN and DR was independently associated with HbA1c variability as assessed retrospectively from HbA1c values obtained during the 2-year period preceding the enrollment. This study assessed DN by albuminuria and the estimated glomerular filtration rate (eGFR), and patients were stratified by chronic kidney disease (CKD) stage or phenotype.

RESEARCH DESIGN AND METHODS

Study cohort
We used the data collected at the baseline visit for the RIACE Italian Multicenter Study (registered with ClinicalTrials.gov, NCT00715481; URL http://clinicaltrials.gov/ct2/show/NCT00715481), an observational, prospective cohort study on the effect of eGFR on morbidity and mortality from cardiovascular disease (CVD) in type 2 diabetes.

The RIACE cohort consisted of 15,933 Caucasian patients with type 2 diabetes (defined by the American Diabetes Association criteria) consecutively attending 19 hospital-based diabetes clinics of the National Health Service throughout Italy (see Supplementary Data) in years 2007–2008. Exclusion criteria were dialysis or renal transplantation. The study protocol was reviewed by the locally appointed ethics boards. The quality and completeness of data were controlled, and 160 patients were excluded due to missing or implausible values. The remaining 15,773 subjects were subsequently analyzed. Multiple HbA1c values (3–5, mean ± SD: 4.52 ± 0.76) serially measured during the 2-year period preceding the enrollment were available from nine centers for 8,290 patients (52.6% of the entire cohort). CVD risk factors and complications were determined as part of the baseline assessment. Measurements were undertaken from a standardized protocol across study centers.

CVD risk factors
All patients underwent a structured interview to collect information on age, smoking status, known diabetes duration, and current glucose-, blood pressure (BP)-, and lipid-lowering treatments as well as antiplatelet and anticoagulant therapy, with indication of the class of drug. Weight and height were assessed, with calculation of BMI. BP was measured with a sphygmomanometer after a 5-min rest. Triglycerides and total and HDL cholesterol were determined by standard analytical methods; LDL cholesterol was calculated by the Friedewald formula. Hypertension was defined as systolic BP ≥140 mmHg and/or diastolic BP ≥90 mmHg and/or antihypertensive treatment. Dyslipidemia was defined as high (≥100 mg/dL) LDL cholesterol and/or lipid-lowering treatment.

Complications
The presence of CKD at baseline was assessed by albuminuria and serum creatinine. As previously reported in detail (14), the albumin excretion rate (AER) was obtained from timed (24 h) urine collections or calculated from the albumin-to-creatinine ratio in early-morning, first-voided urine samples, in the absence of symptoms and signs of urinary tract infection or other interfering clinical conditions. Albuminuria was measured in one to three fresh urine samples for each patient by immunonephelometry or immuno nephelometry, and the geometric mean was used for analysis in case of multiple measurements. In subjects with multiple measurements (≥4, 062 with at least two and 2,310 with three values), the concordance rate between the first value and the geometric mean was >90% for all classes of albuminuria (14). As an external quality control of urinary albumin assays, 50 samples from each center were reanalyzed at the reference laboratory using the immunonephelometry method to verify that the coefficients of variation between the peripheral and the central values were <15% at least in the relevant clinical range of 15–500 mg/L, which was the case for 94% of samples (14). Patients were then assigned to one of the following categories of albuminuria (mg/24 h): normoalbuminuria (AER <30), microalbuminuria (AER 30–299), or macroalbuminuria (AER ≥300).

Serum (and urine) creatinine was measured by the modified Jaffe method. One to three measurements were obtained for each patient, and eGFR was calculated by the four-variable Modification of Diet in Renal Disease study equation (15), using the mean serum creatinine value in case of multiple measures, as reported in previous publications (14,16,17). Patients were then assigned to one of the following categories of eGFR (ml/min/1.73 m²): 1 (≥90), 2 (60–89), 3 (30–59), 4 (15–29), and 5 (<15). Finally, subjects were classified as having no CKD or CKD stages 1–5, based on the presence or absence of micro- or macroalbuminuria, and the value of the eGFR, as calculated by the Modification of Diet in Renal Disease study equation, according to the National Kidney Foundation’s Kidney Disease Outcomes Quality Initiative (18). Patients assigned to CKD stages (and GFR classes) 4 and 5 were pooled. As previously reported (17), CKD patients were further classified as having one of the following CKD phenotypes: albuminuria alone (stages 1–2 CKD), reduced eGFR alone (stage ≥3 CKD without albuminuria), or both (stage ≥3 CKD with albuminuria).

The presence of DR at baseline was assessed by dilated fundoscopy. In each center, an expert ophthalmologist was asked to complete a standardized report form and to classify DR as absent, mild, moderate, or severe nonproliferative DR (NPDR), proliferative DR (PDR), and maculopathy, according to the Global Diabetic Retinopathy Project Group (19). Patients were classified based on the actual fundus appearance or the retinal disease condition that had eventually required a previous photocoagulation or surgical treatment. On the basis of the worst eye, patients with NPDR of mild (microaneurysms only) or moderate (microaneurysms and other microvascular lesions) degree were classified as having nonadvanced DR, whereas those with severe NPDR or pre-PDR (i.e., microaneurysms/hemorrhages in four quadrants, or
venous beading in two quadrants, or intraretinal microvascular abnormalities in one quadrant), PDR (i.e., neovascularization from the disc or from elsewhere, vitreous hemorrhages, or tractional retinal detachment), maculopathy (retinal thickening or hard exudates distant from, approaching, or involving the center of the macula), or blindness (if less than 1/10 normal vision or 20/200 on the Snellen test) were grouped into the advanced DR category (20).

Prevalent CVD at baseline was assessed from medical history by recording previously documented major acute CVD events, including myocardial infarction, stroke, foot ulcer or gangrene, amputation, coronary, carotid, and lower limb revascularization, and surgery for aortic aneurysm. CVD events were adjudicated based on hospital discharge records or specialist visits by an ad hoc committee in each center (21).

**HbA1c variability**

HbA1c was measured in each center by high-performance liquid chromatography using DCCT-aligned methods. Average HbA1c and HbA1c variability was calculated for each patient as the intradividual mean (HbA1c-MEAN) and SD (HbA1c-SD), respectively, for HbA1c values obtained during the 2-year period preceding recruitment, including that obtained at the enrollment. The interindividual difference in the number of HbA1c assessments (a few values would make the SD apparently greater than many values) was adjusted according to the formula: adj-HbA1c-SD = SD/[n/(n-1)] (9,11). Furthermore, as a normalized measure of variability, the coefficient of variation of HbA1c (HbA1c-CV) was calculated as the ratio of HbA1c-SD and HbA1c-MEAN to correct for larger SDs due to higher absolute values of HbA1c-MEAN (10).

**Statistical analysis**

Data are expressed as median (interquartile range [IQR]) and/or mean ± SD for continuous variables and number of subjects and percentage for categorical variables. Patients were stratified by presence and severity of microvascular complications. Continuous variables were compared by the Student t test or one-way ANOVA for normally distributed variables and by Mann-Whitney U test or Kruskal-Wallis test for variables with a skewed distribution. Pearson $\chi^2$ was applied to categorical variables.

Logistic regression analyses with backward variable selection (probability for removal >0.10) were performed to assess whether increments in HbA1c-MEAN (model 1), increments of HbA1c-SD and HbA1c-SD (model 2), and quartiles of both variables (model 3) were independent correlates of microvascular complications compared with no complications. Covariates were age, BMI, sex, known disease duration, smoking habits, triglycerides, HDL cholesterol, hypertension, dyslipidemia, previous major CVD events, specific treatments, and eGFR and albuminuria categories if DR was the dependent variable or DR categories if renal parameters were the dependent variable. Results of these analyses were expressed as odd ratios (ORs) with their 95% CIs. Logistic regression analyses were repeated entering adj-HbA1c-SD (or HbA1c-CV) instead of HbA1c-SD as a measure of HbA1c variability.

All P values were two-sided, and a P value <0.05 was considered statistically significant. Statistical analyses were performed using SPSS 13.0 software (SPSS Inc., Chicago, IL).

**RESULTS**

**Patients’ characteristics**

Participants included in this analysis (i.e., those with 3 to 5 HbA1c values) had a median (IQR) age and duration of diabetes at enrollment of 68 (61–74) and 14 (7–23) years, respectively. The male-to-female ratio was 57:43. Likely due to longer disease duration, these subjects showed a worse CVD risk profile and a higher prevalence of any CVD event and were more frequently receiving treatment with glucose-, lipid-, and BP-lowering drugs than those excluded from the analysis due to unavailability of serial HbA1c measurements (Supplementary Table 1). HbA1c-MEAN of participants was 7.57% (6.86–8.38), HbA1c-SD was 0.46 (0.29–0.74), and adj-HbA1c-SD was 0.40 (0.25–0.65). The variability measures of HbA1c-SD and adj-HbA1c-SD were closely related to HbA1c-MEAN ($r = 0.428$ and $r = 0.434$, respectively, $P < 0.01$ for both). Consistently, HbA1c-SD (and adj-HbA1c-SD) progressively increased throughout HbA1c-MEAN quartiles and vice versa (Supplementary Table 2); likewise, HbA1c-CV progressively increased with HbA1c-MEAN quartiles (Supplementary Table 2) and also with HbA1c-SD quartiles (Supplementary Table 3).

**Average HbA1c and HbA1c variability**

Increasing HbA1c-MEAN was associated with longer diabetes duration and a more adverse CVD risk profile, and subjects were more frequently taking insulin alone or combined with an oral hypoglycemic agent, taking lipid-lowering drugs, and receiving antihypertensive treatment (including inhibitors of the renin-angiotensin system). Prevalence of albuminuria (micro- and macroalbuminuria) and DR (nonadvanced and advanced) increased markedly across HbA1c-MEAN quartiles. Rates of reduced eGFR (<60 mL/min/1.73 m<sup>2</sup>), stages 1–2 CKD, and stages 3–5 albuminuric CKD increased by 49, 68, and 88%, respectively, from the lowest to the highest HbA1c-MEAN quartile, whereas the rate of stages 3–5 nonalbuminuric CKD did not change significantly (Supplementary Table 2).

Higher HbA1c variability (i.e., higher HbA1c-SD) was associated with younger age, lower age at diabetes diagnosis, shorter diabetes duration, higher HbA1c and BMI values, and a more adverse lipid profile, with no differences in BP levels. Prevalence of micro- and macroalbuminuria, reduced eGFR, stages 1–2 CKD, stages 3–5 albuminuric CKD, and advanced DR increased progressively with increasing HbA1c-SD, whereas that of stages 3–5 nonalbuminuric CKD and nonadvanced DR did not change (Supplementary Table 3). Findings were similar for HbA1c-CV, suggesting that differences among HbA1c-SD quartiles were not solely attributable to differences in absolute HbA1c-MEAN values (data not shown).

In Table 1, prevalence rates are given for HbA1c-MEAN and HbA1c-SD above and below the population median values. For micro- and macroalbuminuria, reduced eGFR, stages 1–2 CKD, stages 3–5 albuminuric CKD, and advanced DR, the highest prevalence rates were observed when HbA1c-MEAN and HbA1c-SD were above the median; these subjects also showed the worst CVD risk profile. Conversely, the lowest prevalence for the above microvascular end points was found when both measures were below the median. No differences among the four groups were observed for stages 3–5 nonalbuminuric CKD. Interestingly, patients above the median for HbA1c-MEAN and below the median for HbA1c-SD had similar prevalence rates of albuminuria, reduced eGFR, and CKD phenotypes as patients below the median for HbA1c-MEAN and above the
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Table 1—Main clinical characteristics and prevalence of retinopathy and renal disease in subjects stratified according to HbA1c-MEAN and HbA1c-SD above and below the cohort median values

| Variables                        | HbA1c-MEAN below | HbA1c-MEAN above | P*    |
|----------------------------------|------------------|------------------|-------|
|                                  | HbA1c-SD below   | HbA1c-SD above   |       |
| n (%) ()                       | 2,779 (33.5)     | 1,367 (16.5)     |       |
|                                  | 1,366 (16.5)     | 2,778 (33.5)     |       |
| HbA1c-MEAN (%)                   | 6.70 ± 0.58      | 6.91 ± 0.51      |       |
|                                  | 8.34 ± 0.73      | 8.77 ± 0.98      |       |
| HbA1c-SD (%)                     | 0.27 ± 0.10      | 0.76 ± 0.32      |       |
|                                  | 0.32 ± 0.09      | 0.98 ± 0.56      |       |
| HbA1c-CV (%)                     | 4.08 ± 1.45      | 10.97 ± 4.60     |       |
|                                  | 3.81 ± 1.12      | 11.14 ± 6.10     |       |
| Adj-HbA1c-SD (%)                 | 0.24 ± 0.09      | 0.66 ± 0.27      |       |
|                                  | 0.28 ± 0.08      | 0.86 ± 0.48      |       |
| Males, n (%)                     | 1,627 (58.5)     | 836 (61.2)       |       |
|                                  | 677 (49.6)       | 1,586 (51.7)     | <0.0001 |
| Age (years)                      | 67.4 ± 9.7       | 66.2 ± 10.1      |       |
|                                  | 69.1 ± 9.3       | 66.6 ± 10.2      | <0.0001 |
| Smoking, n (%)                   | 1,480 (53.2)     | 784 (57.4)       |       |
|                                  | 691 (50.5)       | 1,529 (55.0)     |       |
| Never                            | 1,480 (53.2)     | 784 (57.4)       |       |
| Former                           | 911 (32.8)       | 407 (29.8)       |       |
| Current                          | 388 (14.0)       | 175 (12.8)       |       |
| BMI (kg/m²)                      | 28.3 ± 4.7       | 29.0 ± 5.1       |       |
|                                  | 28.5 ± 5.1       | 29.5 ± 5.2       | <0.0001 |
| Triglycerides (mmol/L)           | 0.41 ± 0.80      | 1.54 ± 0.87      |       |
|                                  | 1.52 ± 0.93      | 1.68 ± 1.00      | <0.0001 |
| Cholesterol (mmol/L)             | 4.74 ± 0.90      | 4.73 ± 0.93      |       |
|                                  | 4.77 ± 0.91      | 4.76 ± 0.94      | ns     |
| Dyslipidemia, n (%)              | 2,323 (83.6)     | 1,117 (81.7)     |       |
|                                  | 1,167 (85.4)     | 2,296 (82.6)     |       |
| BP (mmHg)                        | 138.6 ± 17.9     | 138.4 ± 18.0     |       |
| Systolic                         | 138.6 ± 17.9     | 138.4 ± 18.0     |       |
|                                  | 141.6 ± 17.8     | 140.2 ± 19.0     | <0.0001 |
| Diastolic                        | 78.2 ± 8.9       | 78.8 ± 9.2       |       |
|                                  | 78.6 ± 8.9       | 78.6 ± 9.6       | ns     |
| Hypertension, n (%)              | 2,350 (84.6)     | 1,146 (83.8)     |       |
|                                  | 1,229 (90.0)     | 2,383 (85.8)     | <0.0001 |
| Diabetes treatment, n (%)        | 674 (23.3)       | 132 (9.7)        |       |
|                                  | 41 (3.0)         | 60 (2.2)         |       |
| OHA                             | 1,801 (64.8)     | 1,022 (74.8)     |       |
|                                  | 869 (63.6)       | 1,636 (58.9)     |       |
| OHA + insulin                   | 91 (3.3)         | 58 (4.2)         |       |
|                                  | 184 (13.5)       | 442 (15.9)       |       |
| Insulin                         | 240 (8.6)        | 155 (11.3)       |       |
|                                  | 272 (19.9)       | 640 (23.0)       |       |
| Lipid-lowering treatment, n (%)  | 1,376 (49.5)     | 611 (44.7)       |       |
|                                  | 721 (52.8)       | 1,408 (50.7)     | <0.0001 |
| Antihypertensive treatment, n (%)| 2,029 (73.0)     | 971 (71.0)       |       |
|                                  | 1,039 (76.1)     | 2,043 (73.5)     | 0.028  |
| ACE-I/ARB treatment, n (%)       | 1,614 (58.1)     | 789 (57.7)       |       |
|                                  | 873 (63.9)       | 1,715 (61.7)     | <0.0001 |
| Albuminuria, n (%)               | 2,182 (78.9)     | 1,029 (75.3)     |       |
|                                  | 998 (73.1)       | 1,845 (66.4)     |       |
| Normalalbuminuria                | 498 (17.9)       | 284 (20.8)       |       |
|                                  | 305 (22.3)       | 742 (26.7)       | <0.0001 |
| Macroalbuminuria                 | 100 (3.6)        | 54 (4.0)         |       |
|                                  | 63 (4.6)         | 191 (6.9)        | <0.0001 |
| Serum creatinine (μmol/L)        | 84.0 ± 34.5      | 86.6 ± 39.8      |       |
|                                  | 84.0 ± 35.4      | 85.7 ± 31.8      | ns     |
| eGFR, n (%)                      | 730 (26.3)       | 423 (30.9)       |       |
|                                  | 338 (24.7)       | 757 (27.2)       |       |
| $\geq$90 mL/min/1.73 m²          | 1,596 (57.4)     | 675 (49.4)       |       |
|                                  | 733 (53.7)       | 1,396 (50.3)     | <0.0001 |
| 60–89 mL/min/1.73 m²             | 417 (15.0)       | 243 (17.8)       |       |
|                                  | 272 (19.9)       | 569 (20.3)       | <0.0001 |
| 30–59 mL/min/1.73 m²             | 36 (1.3)         | 26 (1.9)         |       |
|                                  | 23 (1.7)         | 56 (2.0)         | 0.194  |
| <30 mL/min/1.73 m²               | 190 (6.8)        | 162 (11.9)       |       |
|                                  | 179 (13.1)       | 319 (11.5)       | 0.013  |
| CKD phenotype, n (%)             | 274 (9.9)        | 162 (11.9)       |       |
|                                  | 179 (13.1)       | 319 (11.5)       | 0.0001 |
| Retinopathy, n (%)               | 179 (6.4)        | 107 (7.8)        |       |
|                                  | 116 (8.5)        | 306 (11.0)       | <0.0001 |

Continued on p. 2305
Variables $\text{HbA1c-SD below}$ $\text{HbA1c-SD above}$

| Groups | HbA1c-MEAN below | HbA1c-MEAN above | P* |
|--------|------------------|------------------|----|
| None   | 2,344 (84.3)     | 1,139 (83.3)     |    |
|        | 889 (65.1)       | 1,897 (68.3)     |    |
| Nonadvanced | 300 (10.8)    | 142 (10.4)       |    |
|        | 322 (23.6)       | 506 (18.2)       | $<0.0001$ |
| Advanced | 135 (4.9)       | 86 (6.3)         |    |
|        | 155 (11.3)       | 375 (13.5)       | $<0.0001$ |

Values are mean ± SD for continuous variables and n (%) for categorical variables. ARB, angiotensin-receptor blocker; OHA, oral hypoglycemic agent. *P values for comparison between quartiles using the one-way ANOVA for parametric or the corresponding Kruskal-Wallis test for nonparametric (triglycerides) continuous variables and the $\chi^2$ test for categorical variables.

CONCLUSIONS—Recent evidence suggests that microvascular complications are predicted not only by HbA1c levels but also by HbA1c variability from one visit to the next, independently of average HbA1c and known risk factors for microangiopathy, both in type 1 (9–11) and type 2 (12,13) diabetes. At variance with these previous reports (9–13), our study covers the entire spectrum of renal disease in diabetic individuals, with the sole exception of end-stage renal disease, and also includes DR, the other microvascular complication, which has not been investigated in type 2 diabetes. This broader analysis provides the first evidence of a wide spectrum of associations of average HbA1c and HbA1c variability with microvascular complications in subjects with type 2 diabetes, thus suggesting that different mechanisms might link glycemic control to microvascular abnormalities in these individuals. In fact, both measures correlated only with microalbuminuria (and stages 1–2 CKD) to the same extent and independently of each other and of known risk factors. In contrast, HbA1c-SD was associated with macroalbuminuria and albuminuric stages 3–5 CKD, independently of HbA1c-MEAN, even after adjustment for other known predictors of DN, whereas HbA1c-MEAN was not. Conversely, HbA1c-SD did not add to HbA1c-MEAN as an independent correlate of both nonadvanced and advanced DR. Finally, neither HbA1c-MEAN nor HbA1c-SD was independently associated with reduced eGFR and nonalbuminuric stages 3–5 CKD.

Concerning microalbuminuria, our data support two recent analyses of individuals with type 2 diabetes from Japan (12) and Taiwan (13), where HbA1c variability predicted the development of microalbuminuria independently of average HbA1c in a mean follow-up period of 4.3 and 6.2 years, respectively. Our results are also in accordance with previous reports in subjects with type 1 diabetes (9–11). In particular, Kaplan-Meier survival curves in the FinnDiane Study (10) demonstrated that patients above the median for HbA1c-MEAN and below the median for HbA1c-SD had similar rate of progression in renal status (as defined as a shift to a higher albuminuria level or to end-stage renal disease) as patients below the median for HbA1c-MEAN and above the median for HbA1c-SD. This is consistent with our finding that groups discordant for below and above median values of HbA1c-MEAN and HbA1c-SD showed similar rates of microalbuminuria (and stages 1–2 CKD), suggesting a distinct but equally important effect of both average HbA1c and HbA1c variability.

Concerning other DN markers and CKD phenotypes, although rates of microalbuminuria, reduced eGFR, and stages 3–5 albuminuric CKD were also similar between subjects above the median for HbA1c-MEAN and below the median for HbA1c-SD and those vice versa, logistic regression analysis showed that only HbA1c-SD was independently associated with microalbuminuria and stages 3–5 albuminuric CKD, whereas neither HbA1c-MEAN nor HbA1c-SD correlated with reduced eGFR or stages 3–5 nonalbuminuric CKD, although the highest HbA1c-SD quartiles did. Altogether, these results suggest that HbA1c variability might be even more important than average HbA1c in conferring overall DN risk; however, longitudinal studies are needed to clarify this issue. Moreover, these data confirm (and extend to HbA1c variability) our previous observation that reduced eGFR and, particularly, the nonalbuminuric CKD phenotype, in which an eGFR <60 mL/min/1.73 m$^2$ develops in the absence of albuminuria, are not related to glycemic control (17). This further supports the concept that macroangiopathy,
rather than microangiopathy, is the prevailing renal pathology underlying nonalbuminuric CKD (17), which nowadays is the predominant form of renal impairment in subjects with type 2 diabetes (17,22–24).

Concerning DR, our study showed that only the rate of advanced DR increased significantly with increasing HbA1c variability and that no effect of HbA1c-SD could be detected on nonadvanced or advanced DR when adjusting for HbA1c-MEAN and other known predictors of DR. This is at variance with findings in subjects with type 1 diabetes showing that increasing HbA1c variability adds to the risk of DR exceeding that predicted by average HbA1c, alone (9,10). This discrepancy has no obvious explanation, especially if we consider that, again in type 1 diabetes, a rapid improvement of glycemic control can lead to a short-term worsening of DR, followed by a net improvement in the long-term (25), which could be lost if another HbA1c increment ensues. It might be speculated that HbA1c variability is of lower magnitude in subjects with type 2 diabetes and, hence, its effect is masked by that of average HbA1c and possibly of other variables related to Table 2—Logistic regression analysis with backward variable selection of independent correlates of micro- and macroalbuminuria, and eGFR <60 mL/min/1.73 m² versus normoalbuminuria and, respectively, eGFR ≥60 mL/min/1.73 m²

| Variables                  | Microalbuminuria | P   | Macroalbuminuria | P          | eGFR <60 mL/min/1.73 m² | P   |
|----------------------------|------------------|-----|------------------|------------|------------------------|-----|
| Age, × year                | 1.029 (1.022–1.035) | 0.0001 | 1.031 (1.017–1.044) | 0.0001 | 1.101 (1.092–1.109) | 0.0001 |
| Diabetes duration, × year  | 1.013 (1.001–1.026) | 0.032 |                   |            |                        |     |
| Gender, male               | 2.189 (1.938–2.473) | 0.0001 | 2.746 (2.137–3.530) | 0.0001 | 0.447 (0.391–0.511) | 0.0001 |
| BMI × unit                 | 1.028 (1.016–1.040) | 0.0001 | 1.049 (1.026–1.072) | 0.0001 | 1.028 (1.015–1.042) | 0.0001 |
| Smoking                    |                   |       |                   |            |                        |     |
| Never                      | 1.0               |     | 1.0               |            |                        |     |
| Former                     | 1.026 (0.904–1.165) | 0.690 | 1.185 (0.922–1.523) | 0.185 |                       |     |
| Current                    | 1.387 (1.182–1.626) | 0.0001 | 2.043 (1.519–2.749) | 0.0001 |                       |     |
| Triglycerides, × 0.0113 mmol/L | 1.002 (1.002–1.003) | 0.0001 | 1.005 (1.004–1.006) | 0.0001 | 1.003 (1.002–1.004) | 0.0001 |
| HDL cholesterol, × 0.259 mmol/L |                   |       | 0.984 (0.979–0.989) | 0.0001 |                       |     |
| Hypertension               | 1.981 (1.632–2.405) | 0.0001 | 5.329 (2.801–10.140) | 0.0001 | 1.551 (1.219–1.974) | 0.0001 |
| Previous CVD event         | 1.213 (1.073–1.372) | 0.002 | 1.381 (1.102–1.732) | 0.005 | 1.772 (1.552–2.024) | 0.0001 |
| Diabetes treatment         |                   |       |                   |            |                        |     |
| Diet                       | 1.0               | 0.020 | 1.0               | 0.0001 | 1.0                    | 0.0001 |
| OHA                        | 1.151 (0.939–1.411) | 0.177 | 1.247 (0.770–2.021) | 0.369 | 0.830 (0.667–1.034) | 0.096 |
| OHA + insulin              | 1.198 (0.913–1.573) | 0.192 | 1.792 (1.018–3.156) | 0.043 | 0.852 (0.637–1.139) | 0.280 |
| Insulin                    | 1.422 (1.114–1.816) | 0.005 | 2.892 (1.719–4.864) | 0.0001 | 1.913 (1.492–2.453) | 0.0001 |
| Albuminuria                |                   |       |                   |            |                        |     |
| Normoalbuminuria           | 1.0               |     |                  |            |                        |     |
| Microalbuminuria           | 1.619 (1.404–1.868) | 0.0001 |                       |            |                        |     |
| Macroalbuminuria           | 5.306 (4.154–6.778) | 0.0001 |                       |            |                        |     |
| Retinopathy                |                   |       |                   |            |                        |     |
| None                       | 1.0               | 0.0001 | 1.0               | 0.0001 | 1.0                    | 0.0001 |
| Nonadvanced                | 1.396 (1.204–1.619) | 0.0001 | 1.757 (1.324–2.331) | 0.0001 | 1.213 (1.029–1.432) | 0.022 |
| Advanced                   | 1.901 (1.579–2.827) | 0.0001 | 3.782 (2.816–5.079) | 0.0001 | 1.582 (1.294–1.934) | 0.0001 |
| Model 1                    |                   |       |                   |            |                        |     |
| HbA1c-MEAN, 1% increment   | 1.159 (1.103–1.218) | 0.0001 | 1.094 (0.996–1.202) | 0.059 |                       |     |
| Model 2                    |                   |       |                   |            |                        |     |
| HbA1c-MEAN, 1% increment   | 1.117 (1.058–1.179) | 0.0001 |                       |            | 0.944 (0.888–1.003) | 0.062 |
| HbA1c-SD, 1% increment     | 1.249 (1.105–1.410) | 0.0001 | 1.348 (1.086–1.674) | 0.007 | 1.151 (0.998–1.327) | 0.053 |
| Model 3                    |                   |       |                   |            |                        |     |
| HbA1c-MEAN quartiles       |                   | 0.001 |                   |            |                        |     |
| Quartile 1                 | 1.0               |     |                  |            |                        |     |
| Quartile 2                 | 1.018 (0.863–1.201) | 0.823 |                        |            |                        |     |
| Quartile 3                 | 1.049 (0.885–1.245) | 0.581 |                        |            |                        |     |
| Quartile 4                 | 1.353 (1.129–1.621) | 0.001 |                        |            |                        |     |
| HbA1c-SD quartiles         |                   | 0.008 |                   | 0.033     | 0.0001                 | 0.023 |
| Quartile 1                 | 1.0               |     |                  |            |                        |     |
| Quartile 2                 | 1.033 (0.878–1.217) | 0.693 | 0.939 (0.672–1.312) | 0.712 | 1.003 (0.838–1.201) | 0.971 |
| Quartile 3                 | 1.142 (0.968–1.347) | 0.115 | 1.044 (0.757–1.440) | 0.792 | 1.233 (1.028–1.480) | 0.024 |
| Quartile 4                 | 1.310 (1.102–1.558) | 0.002 | 1.410 (1.031–1.929) | 0.032 | 1.242 (1.022–1.510) | 0.030 |

ORs of variables except HbA1c-MEAN and HbA1c-SD and their quartiles were determined by multivariate logistic analysis from model 1. The results did not change significantly in model 2 and model 3. Other variables not in equation: dyslipidemia. OHA, oral hypoglycemic agent.
**Table 3**—Logistic regression analysis with backward variable selection of independent correlates of stages 1–2 CKD, stages 3–5 nonalbuminuric CKD, and stages 3–5 albuminuric CKD versus no CKD

| Variables                                      | Stages 1–2 CKD | Stages 3–5 nonalbuminuric CKD | Stages 3–5 albuminuric CKD |
|------------------------------------------------|----------------|--------------------------------|----------------------------|
| Age, × year                                    | 1.026 (1.019–1.033) | 0.0001 | 1.113 (1.102–1.124) | 0.0001 | 1.107 (1.093–1.121) | 0.0001 |
| Diabetes duration, × year                      |                |                                |                            |
| Gender, male                                   | 2.472 (2.152–2.841) | 0.0001 | 0.434 (0.367–0.513) | 0.0001 | 1.194 (0.977–1.459) | 0.083 |
| BMI, × unit                                    | 1.025 (1.022–1.048) | 0.0001 | 1.038 (1.022–1.055) | 0.0001 | 1.052 (1.032–1.072) | 0.0001 |
| Smoking                                        |                |                                |                            |
| Previous CVD event                             |                |                                |                            |
| Hypertension                                   | 2.222 (1.804–2.736) | 0.0001 | 1.539 (1.163–2.036) | 0.003 | 2.878 (1.905–4.347) | 0.0001 |
| HDL cholesterol, × 0.259 mmol/L               | 1.003 (1.002–1.003) | 0.0001 | 1.003 (1.002–1.004) | 0.0001 | 1.006 (1.004–1.007) | 0.0001 |
| Triglycerides, × 0.0113 mmol/L                 | 1.983 (0.977–0.990) | 0.0001 | 0.985 (0.977–0.993) | 0.0001 |                                  |                            |
| Obesity                                        |                |                                |                            |
| Smoking                                        | 0.0001 |                                |                            |
| Diabetes treatment                             | 0.020 | 0.0001 |                                | 0.0001 |                                | 0.0001 |
| Diet                                           |                |                                |                            |
| OHA                                            | 1.260 (1.006–1.579) | 0.045 | 0.933 (0.715–1.217) | 0.609 | 0.943 (0.671–1.326) | 0.737 |
| OHA + insulin                                  | 1.468 (1.092–1.975) | 0.011 | 1.150 (0.802–1.647) | 0.447 | 0.958 (0.613–1.497) | 0.851 |
| Insulin                                        | 1.379 (1.042–1.824) | 0.025 | 1.881 (1.377–2.569) | 0.0001 | 3.279 (2.266–4.746) | 0.0001 |
| Retinopathy                                    |                |                                |                            |
| Current                                        | 1.046 (1.187–1.666) | 0.0001 |                                | 1.514 (1.153–1.988) | 0.003 |
| Current                                        | 1.040 (1.057–1.102) | 0.0001 |                                | 1.038 (1.022–1.055) | 0.0001 |
| Diet                                           |                |                                |                            |
| OHA                                            | 1.260 (1.006–1.579) | 0.045 | 0.933 (0.715–1.217) | 0.609 | 0.943 (0.671–1.326) | 0.737 |
| OHA + insulin                                  | 1.468 (1.092–1.975) | 0.011 | 1.150 (0.802–1.647) | 0.447 | 0.958 (0.613–1.497) | 0.851 |
| Current                                        | 1.046 (1.187–1.666) | 0.0001 |                                | 1.514 (1.153–1.988) | 0.003 |
| Current                                        | 1.040 (1.057–1.102) | 0.0001 |                                | 1.038 (1.022–1.055) | 0.0001 |

ORs of variables except HbA1c-MEAN and HbA1c-SD and their quartiles were determined by multivariate logistic analysis from model 1. The results did not change significantly in model 2 and model 3. Other variables not in equation: dyslipidemia. OHA, oral hypoglycemic agent.

glycemic exposure, such as diabetes duration and treatments.

In addition to showing that the effect of HbA1c variability (and of average HbA1c) on microvascular complications in not univocal, our study confirms the results of previous reports indicating that HbA1c change from one visit to the next affects the risk of (albuminuric) DN (9–13). In fact, although the ranges of HbA1c-MEAN and HbA1c-SD were different in all these studies, the effect of HbA1c variability was at least as much as that of average HbA1c, albeit not predominant, except in our study showing a higher impact of HbA1c-SD on macroalbuminuria and to a lesser extent on reduced eGFR and the combination of the two abnormalities in stages 3–5 albuminuric CKD. This suggests that HbA1c variability is a major risk factor for the development of DN, at least of the albuminuric forms, although the underlying mechanisms have not been clarified yet (4).

One possible explanation is that even periods of sustained hyperglycemia are “remembered,” thus conferring an increased risk of microvascular complications (26), and, hence, that the detrimental effect of HbA1c variability may be mediated through the same mechanism underlying the “metabolic memory” phenomenon, including oxidative stress (27). Indeed, in patients with type 2 diabetes, overproduction of reactive oxygen species was associated with short-term glycemic excursions rather than with sustained hyperglycemia (28), although there are data showing a prooxidant effect of longer period of hyperglycemia (29,30).

Another possible mechanism is that, because the risk of microvascular complications increases exponentially as HbA1c rises (2), subjects with higher HbA1c variability would “accumulate” a surplus of risk
in the periods spent at the upper end of their HbA1c range. This hypothesis might be indirectly supported by our observation that the effect of HbA1c variability is a statistically significant effect in the higher quartile of HbA1c-SD.

Finally, the link between fluctuations of HbA1c and risk of microvascular complications might relate to the fact that patients with a higher HbA1c-SD are those with a worse CVD risk profile and a more intensive glucose-lowering treatment. However, multiple regression analyses showed that the association of HbA1c-SD with microvascular and, particularly, DN parameters was independent of confounding factors, including the higher BMI and triglycerides and lower HDL cholesterol levels that characterize the metabolic syndrome, a condition associated with an increased risk of developing renal disease (31).

Strengths of this study include the large size of the cohort, the completeness of data, the analysis of a contemporary dataset, the adjustment for treatments, and, as mentioned above, the concurrent analysis of DR and DN, the latter assessed as both albuminuria and reduced eGFR. The main limitation is the cross-sectional design for the assessment of DN and DR that did not allow us to examine the effect of HbA1c variability on the development of microvascular complications in uncomplicated individuals, as in the studies of Sugawara et al. (12) and Hsu et al. (13).

Another limitation might be that the RIACE participants who had serial (3–5) HbA1c measures had a longer diabetes duration, a worse CVD risk profile, a higher prevalence of any CVD event, and a higher rate of treatment than those who did not and were therefore excluded from this analysis. However, virtually all subjects from the nine centers that made available these data had more than two HbA1c measures, independently of their HbA1c variability, although a selection bias cannot be ruled out conclusively.

Other possible limitations concerning HbA1c values are that they were performed in each center as a part of the patient’s standard care, with no prespecified

### Table 4—Logistic regression analysis with backward variable selection of independent correlates of nonadvanced and advanced diabetic retinopathy versus no retinopathy

| Variables                      | OR (95% CI) | P       | OR (95% CI) | P       |
|--------------------------------|-------------|---------|-------------|---------|
|                                | Nonadvanced retinopathy | Advanced retinopathy |
| Age, × year                    | 0.986 (0.979–0.994) | 0.0001  | 0.957 (0.947–0.967) | 0.0001  |
| Diabetes duration, × year      | 1.055 (1.047–1.062) | 0.0001  | 1.052 (1.042–1.062) | 0.0001  |
| Smoking                        |              |         |             |         |
| Never                          |              |         |             |         |
| Former                         |              |         |             |         |
| Current                        |              |         |             |         |
| Hypertension                   |              |         |             |         |
| Previous CVD event             |              |         |             |         |
| Diabetes treatment             |              |         |             |         |
| Diet                           |              |         |             |         |
| OHA                            |              |         |             |         |
| OHA + insulin                  |              |         |             |         |
| Insulin                        |              |         |             |         |
| Albuminuria                    |              |         |             |         |
| Normoalbuminuria               | 1.0          |         | 1.0         |         |
| Microalbuminuria               |              |         |             |         |
| Macroalbuminuria               | 1.0          |         | 1.0         |         |
| eGFR                           |              |         |             |         |
| >90 mL/min/1.73 m²             |              |         |             |         |
| 60–89 mL/min/1.73 m²           |              |         |             |         |
| 30–59 mL/min/1.73 m²           |              |         |             |         |
| <30 mL/min/1.73 m²             |              |         |             |         |
| Model 1                        |              |         |             |         |
| HbA1c-MEAN, 1% increment       | 1.236 (1.167–1.308) | 0.0001  | 1.263 (1.178–1.354) | 0.0001  |
| Model 2                        |              |         |             |         |
| HbA1c-MEAN, 1% increment       | 1.326 (1.245–1.412) | 0.0001  | 1.263 (1.178–1.354) | 0.0001  |
| HbA1c-SD 1% increment          | 0.917 (0.758–1.110) | 0.093   |             |         |
| Model 3                        |              |         |             |         |
| HbA1c-MEAN quartiles           |              |         |             |         |
| Quartile 1                     | 1.0          |         | 1.0         |         |
| Quartile 2                     | 1.251 (1.012–1.547) | 0.039   | 0.976 (0.976–1.308) | 0.868   |
| Quartile 3                     | 1.684 (1.365–2.078) | 0.0001  | 1.244 (0.942–1.643) | 0.124   |
| Quartile 4                     | 2.314 (1.852–2.890) | 0.0001  | 1.950 (1.495–2.544) | 0.0001  |
| HbA1c-SD quartiles             |              |         |             |         |

ORs of variables except HbA1c-MEAN and HbA1c-SD and their quartiles were determined by multivariate logistic analysis from model 1. The results did not change significantly in model 2 and model 3. Other variables not in equation: gender, HDL cholesterol, triglycerides, BMI, and dyslipidemia. OHA, oral hypoglycemic agent.
intervals between HbA1c measurements, and that the number of measures per individual varied from 3 to 5. However, noncentralized measurements did not affect intrapatient variability, intervals between measurements ranged from 6 to 9 months, and adj-HbA1c–SD was used to account for difference in the number of measures. Furthermore, although the number of measurements was not as large as in the study of Sugawara et al. (12) and the period analyzed was not as long as in the study of Hsu et al. (13), reanalyses of these surveys showed that using 3-month measures. Furthermore, although the number of measurements was not as large as in the study of Sugawara et al. (12) and the period analyzed was not as long as in the study of Hsu et al. (13), reanalyses of these surveys showed that using 3-month

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