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

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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.

Diabetes Care 36:2301–2310, 2013

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

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Received 2 November 2012 and accepted 4 January 2013.
DOI: 10.2337/dc12-2264. Clinical trial reg. no. NCT00715481, clinicaltrials.gov.

risk factor for the development of diabetic retinopathy (DR) and nephropathy (DN) in individuals with type 1 diabetes. Moreover, HbA_{1c} variability was shown to be an independent variable that added to the effect of HbA_{1c} 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 HbA_{1c} 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 HbA_{1c} variability as assessed retrospectively from HbA_{1c} 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 HbA_{1c} 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 immunoturbidimetry, 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 m^2): 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 funduscopy. 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 beadings 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).

HbA_{1c} variability

HbA_{1c} was measured in each center by high-performance liquid chromatography using DCCT-aligned methods. Average HbA_{1c} and HbA_{1c} variability was calculated for each patient as the intraindividual mean (HbA_{1c}-MEAN) and SD (HbA_{1c}-SD), respectively, for HbA_{1c} values obtained during the 2-year period preceding recruitment, including that obtained at the enrollment. The interindividual difference in the number of HbA_{1c} assessments (a few values would make the SD apparently greater than many values) was adjusted according to the formula: adj-HbA_{1c}-SD = $SD/\sqrt{[n/(n-1)]}$ (9,11). Furthermore, as a normalized measure of variability, the coefficient of variation of HbA_{1c} (HbA_{1c}-CV) was calculated as the ratio of HbA_{1c}-SD and HbA_{1c}-MEAN to correct for larger SDs due to higher absolute values of HbA_{1c}-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 χ^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 HbA_{1c}-MEAN (model 1), increments of HbA_{1c}-MEAN and HbA_{1c}-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-HbA_{1c}-SD (or HbA_{1c}-CV) instead of HbA_{1c}-SD as a measure of HbA_{1c} 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 HbA_{1c} 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 HbA_{1c} measurements (Supplementary Table 1). HbA_{1c}-MEAN of participants was 7.57% (6.86–8.38), HbA_{1c}-SD was 0.46 (0.29–0.74), and adj-HbA_{1c}-SD was 0.40 (0.25–0.65). The variability measures of HbA_{1c}-SD and adj-HbA_{1c}-SD were closely related to HbA_{1c}-MEAN (r = 0.428 and r = 434, respectively, $P <$ 0.01 for both). Consistently, HbA_{1c}-SD (and adj-HbA_{1c}-SD) progressively increased throughout HbA_{1c}-MEAN quartiles and vice versa (Supplementary Table 2); likewise, HbA_{1c}-CV progressively increased with HbA_{1c}-MEAN quartiles (Supplementary Table 2) and also with HbA_{1c}-SD quartiles (Supplementary Table 3).

Average HbA_{1c} and HbA_{1c} variability

Increasing HbA_{1c}-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 HbA_{1c}-MEAN quartiles. Rates of reduced eGFR (<60 mL/min/1.73m^2), stages 1–2 CKD, and stages 3–5 albuminuric CKD increased by 49, 68, and 88%, respectively, from the lowest to the highest HbA_{1c}-MEAN quartile, whereas the rate of stages 3–5 nonalbuminuric CKD did not change significantly (Supplementary Table 2).

Higher HbA_{1c} variability (i.e., higher HbA_{1c}-SD) was associated with younger age, lower age at diabetes diagnosis, shorter diabetes duration, higher HbA_{1c} 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 HbA_{1c}-SD, whereas that of stages 3–5 nonalbuminuric CKD and nonadvanced DR did not change (Supplementary Table 3). Findings were similar for HbA_{1c}-CV, suggesting that differences among HbA_{1c}-SD quartiles were not solely attributable to differences in absolute HbA_{1c}-MEAN values (data not shown).

In Table 1, prevalence rates are given for HbA_{1c}-MEAN and HbA_{1c}-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 HbA_{1c}-MEAN and HbA_{1c}-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 HbA_{1c}-MEAN and below the median for HbA_{1c}-SD had similar prevalence rates of albuminuria, reduced eGFR, and CKD phenotypes as patients below the median for HbA_{1c}-MEAN and above the

Table 1—*Main clinical characteristics and prevalence of retinopathy and renal disease in subjects stratified according to HbA_{1c}-MEAN and HbA_{1c}-SD above and below the cohort median values*

	Groups				
	HbA_{1c}-MEAN below		HbA_{1c}-MEAN above		
Variables	HbA_{1c}-SD below	HbA_{1c}-SD above	HbA_{1c}-SD below	HbA_{1c}-SD above	*P**
n (%)	2,779 (33.5)	1,367 (16.5)	1,366 (16.5)	2,778 (33.5)	
HbA_{1c}-MEAN (%)	6.70 ± 0.58	6.91 ± 0.51	8.34 ± 0.73	8.77 ± 0.98	
HbA_{1c}-SD (%)	0.27 ± 0.10	0.76 ± 0.32	0.32 ± 0.09	0.98 ± 0.56	
HbA_{1c}-CV	4.08 ± 1.45	10.97 ± 4.60	3.81 ± 1.12	11.14 ± 6.10	
Adj-HbA_{1c}-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 (57.1)	<0.0001
Age (years)	67.4 ± 9.7	66.2 ± 10.1	69.1 ± 9.3	66.6 ± 10.2	<0.0001
At diabetes diagnosis	53.5 ± 10.5	54.2 ± 10.8	49.6 ± 10.4	50.0 ± 10.4	<0.0001
Diabetes duration (years)	14.0 ± 10.2	12.0 ± 9.6	19.4 ± 9.6	16.6 ± 10.0	<0.0001
Smoking, *n* (%)					<0.0001
Never	1,480 (53.2)	784 (57.4)	691 (50.5)	1,529 (55.0)	
Former	911 (32.8)	407 (29.8)	451 (33.0)	787 (28.3)	
Current	388 (14.0)	175 (12.8)	225 (16.5)	462 (16.6)	
BMI (kg/m^2)	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)					
Total	4.74 ± 0.90	4.73 ± 0.93	4.77 ± 0.91	4.76 ± 0.94	ns
HDL					
Males	1.28 ± 0.34	1.21 ± 0.32	1.24 ± 0.30	1.19 ± 0.31	<0.0001
Females	1.43 ± 0.37	1.39 ± 0.36	1.40 ± 0.36	1.35 ± 0.36	<0.0001
LDL	2.77 ± 0.77	2.75 ± 0.82	2.76 ± 0.78	2.76 ± 0.85	ns
Non-HDL	3.40 ± 0.85	3.44 ± 0.90	3.45 ± 0.88	3.51 ± 0.96	<0.0001
Dyslipidemia, *n* (%)	2,323 (83.6)	1,117 (81.7)	1,167 (85.4)	2,296 (82.6)	ns
BP (mmHg)					
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* (%)					<0.0001
Diet	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* (%)					<0.0001
Normoalbuminuria	2,182 (78.5)	1,029 (75.3)	998 (73.1)	1,845 (66.4)	
Microalbuminuria	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* (%)					<0.0001
≥90 mL/min/1.73 m^2	730 (26.3)	423 (30.9)	338 (24.7)	757 (27.2)	
60–89 mL/min/1.73 m^2	1,596 (57.4)	675 (49.4)	733 (53.7)	1,396 (50.3)	<0.0001
30–59 mL/min/1.73 m^2	417 (15.0)	243 (17.8)	272 (19.9)	569 (20.5)	<0.0001
<30 mL/min/1.73 m^2	36 (1.3)	26 (1.9)	23 (1.7)	56 (2.0)	0.194
CKD phenotype, *n* (%)					<0.0001
No CKD	1,907 (68.6)	867 (63.4)	819 (60.0)	1,526 (54.9)	
Stages 1–2 CKD	419 (15.1)	231 (16.9)	252 (18.4)	627 (22.6)	<0.0001
Stage ≥3 CKD					
Without albuminuria	274 (9.9)	162 (11.9)	179 (13.1)	319 (11.5)	0.013
With albuminuria	179 (6.4)	107 (7.8)	116 (8.5)	306 (11.0)	<0.0001
Retinopathy, *n* (%)					<0.0001

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Table 1—*Continued*

Variables	Groups				
	HbA_{1c}-MEAN below		HbA_{1c}-MEAN above		
	HbA_{1c}-SD below	HbA_{1c}-SD above	HbA_{1c}-SD below	HbA_{1c}-SD 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 χ^2 test for categorical variables.

median for HbA_{1c}-SD, suggesting distinct but comparable effects of average HbA_{1c} and HbA_{1c} variability. This finding was not observed for nonadvanced and advanced DR. Indeed, DR was more frequent in subjects with HbA_{1c}-MEAN above the median irrespective of HbA_{1c}-SD levels.

Multiple logistic regression models

Because of several potential confounding factors for the association between HbA_{1c} variability and prevalence of microvascular complications, we used logistic regression as a multivariate analysis. Even after adjusting for several CVD risk factors and also for DR, HbA_{1c}-SD was a significant correlate of microalbuminuria, even independently of HbA_{1c}-MEAN. Moreover, HbA_{1c}-SD was also an independent predictor of macroalbuminuria and reduced eGFR, whereas HbA_{1c}-MEAN was not (Table 2). Interestingly, whereas HbA_{1c}-MEAN and HbA_{1c}-SD were both independent correlates of stages 1–2 CKD and only HbA_{1c}-SD was an independent predictor of stages 3–5 albuminuric CKD, neither HbA_{1c}-MEAN nor HbA_{1c}-SD was independently associated with stages 3–5 nonalbuminuric CKD (Table 3). On the contrary, although HbA_{1c}-MEAN was associated with both nonadvanced and advanced DR independently of several variables, including albuminuria (nonadvanced DR) or both albuminuria and eGFR (advanced DR), HbA_{1c}-SD was not an independent correlate of DR (Table 4). Where both HbA_{1c}-MEAN and HbA_{1c}-SD entered as independent correlates in model 3 logistic regression, the ORs of these two variables were quite similar, reaching statistically significant effects in the highest quartile. In all models, expressing HbA_{1c} variability as adj-HbA_{1c}-SD or HbA_{1c}-CV instead of HbA_{1c}-SD did not change the association with risk of microvascular complications.

CONCLUSIONS—Recent evidence suggests that microvascular complications are predicted not only by HbA_{1c} levels but also by HbA_{1c} variability from one visit to the next, independently of average HbA_{1c} 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 HbA_{1c} and HbA_{1c} 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, HbA_{1c}-SD was associated with macroalbuminuria and albuminuric stages 3–5 CKD, independently of HbA_{1c}-MEAN, even after adjustment for other known predictors of DN, whereas HbA_{1c}-MEAN was not. Conversely, HbA_{1c}-SD did not add to HbA_{1c}-MEAN as an independent correlate of both nonadvanced and advanced DR. Finally, neither HbA_{1c}-MEAN nor HbA_{1c}-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 HbA_{1c} variability predicted the development of microalbuminuria independently of average HbA_{1c} 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 HbA_{1c}-MEAN and below the median for HbA_{1c}-SD had similar rate of any 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 HbA_{1c}-MEAN and above the median for HbA_{1c}-SD. This is consistent with our finding that groups discordant for below and above median values of HbA_{1c}-MEAN and HbA_{1c}-SD showed similar rates of microalbuminuria (and stages 1–2 CKD), suggesting a distinct but equally important effect of both average HbA_{1c} and HbA_{1c} variability.

Concerning other DN markers and CKD phenotypes, although rates of macroalbuminuria, reduced eGFR, and stages 3–5 albuminuric CKD were also similar between subjects above the median for HbA_{1c}-MEAN and below the median for HbA_{1c}-SD and those vice versa, logistic regression analysis showed that only HbA_{1c}-SD was independently associated with macroalbuminuria and stages 3–5 albuminuric CKD, whereas neither HbA_{1c}-MEAN nor HbA_{1c}-SD correlated with reduced eGFR or stages 3–5 nonalbuminuric CKD, although the highest HbA_{1c}-SD quartiles did. Altogether, these results suggest that HbA_{1c} variability might be even more important than average HbA_{1c} in conferring overall DN risk; however, longitudinal studies are needed to clarify this issue. Moreover, these data confirm (and extend to HbA_{1c} 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,

Table 2—*Logistic regression analysis with backward variable selection of independent correlates of micro- and macroalbuminuria, and eGFR <60 mL/min/1.73 m^2 versus normoalbuminuria and, respectively, eGFR ≥60 mL/min/1.73 m^2*

	Microalbuminuria		Macroalbuminuria		eGFR <60 mL/min/1.73 m^2	
Variables	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*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		0.0001		0.0001		
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		0.020		0.0001		0.0001
Diet	1.0		1.0		1.0	
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						0.0001
Normoalbuminuria					1.0	
Microalbuminuria					1.619 (1.404–1.868)	0.0001
Macroalbuminuria					5.306 (4.154–6.778)	0.0001
Retinopathy		0.0001		0.0001		0.0001
None	1.0		1.0		1.0	
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 (1.579–2.287)	0.0001	3.782 (2.816–5.079)	0.0001	1.582 (1.294–1.934)	0.0001
Model 1						
HbA_{1c}-MEAN, 1% increment	1.159 (1.103–1.218)	0.0001	1.094 (0.996–1.202)	0.059		
Model 2						
HbA_{1c}-MEAN, 1% increment	1.117 (1.058–1.179)	0.0001			0.944 (0.888–1.003)	0.062
HbA_{1c}-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						
HbA_{1c}-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				
HbA_{1c}-SD quartiles		0.008		0.033		0.023
Quartile 1	1.0				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 HbA_{1c}-MEAN and HbA_{1c}-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.

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 HbA_{1c} variability and that no effect of HbA_{1c}-SD could be detected on nonadvanced or advanced DR when adjusting for HbA_{1c}-MEAN and other known predictors of DR. This is at variance with findings in subjects with type 1 diabetes showing that increasing HbA_{1c} variability adds to the risk of DR exceeding that predicted by average HbA_{1c} 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 HbA_{1c} increment ensues. It might be speculated that HbA_{1c} variability is of lower magnitude in subjects with type 2 diabetes and, hence, its effect is masked by that of average HbA_{1c} and possibly of other variables related to

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*

	Stages 1–2 CKD		Stages 3–5 nonalbuminuric CKD		Stages 3–5 albumunuric CKD	
Variables	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*
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					1.010 (1.000–1.020)	0.046
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		0.0001				0.007
Never	1.0				1.0	
Former	0.981 (0.853–1.129)	0.792			1.233 (1.003–1.516)	0.047
Current	1.406 (1.187–1.666)	0.0001			1.514 (1.153–1.988)	0.003
Triglycerides, × 0.0113 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
HDL cholesterol, × 0.259 mmol/L			0.983 (0.977–0.990)	0.0001	0.985 (0.977–0.993)	0.0001
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
Previous CVD event	1.138 (0.989–1.309)	0.071	1.802 (1.520–2.138)	0.0001	2.044 (1.694–2.466)	0.0001
Diabetes treatment		0.020		0.0001		0.0001
Diet	1.0		1.0		1.0	
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		0.0001		0.010		0.0001
None	1.0		1.0		1.0	
Nonadvanced	1.251 (1.058–1.479)	0.009	1.040 (0.835–1.296)	0.725	2.072 (1.646–2.608)	0.0001
Advanced	2.055 (1.670–2.530)	0.0001	1.539 (1.163–2.036)	0.003	3.877 (2.950–5.096)	0.0001
Model 1						
HbA_{1c}-MEAN, 1% increment	1.196 (1.132–1.264)	0.0001				
Model 2						
HbA_{1c}-MEAN, 1% increment	1.156 (1.089–1.228)	0.0001				
HbA_{1c}-SD, 1% increment	1.221 (1.069–1.395)	0.003			1.331 (1.097–1.613)	0.004
Model 3						
HbA_{1c}-MEAN quartiles		0.0001				
Quartile 1	1.0					
Quartile 2	1.119 (0.933–1.341)	0.227				
Quartile 3	1.140 (0.944–1.377)	0.175				
Quartile 4	1.523 (1.244–1.864)	0.0001				
HbA_{1c}-SD quartiles		0.043				0.006
Quartile 1	1.0				1.0	
Quartile 2	1.082 (0.905–1.294)	0.387			0.934 (0.711–1.228)	0.626
Quartile 3	1.139 (0.949–1.368)	0.162			1.268 (0.968–1.661)	0.084
Quartile 4	1.305 (1.080–1.578)	0.0006			1.469 (1.100–1.962)	0.009

ORs of variables except HbA_{1c}-MEAN and HbA_{1c}-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 HbA_{1c} variability (and of average HbA_{1c}) on microvascular complications in not univocal, our study confirms the results of previous reports indicating that HbA_{1c} change from one visit to the next affects the risk of (albuminuric) DN (9–13). In fact, although the ranges of HbA_{1c}-MEAN and HbA_{1c}-SD were different in all these studies, the effect of HbA_{1c} variability was at least as much as that of average HbA_{1c}, albeit not predominant, except in our study showing a higher impact of HbA_{1c}-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 HbA_{1c} 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 HbA_{1c} 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 HbA_{1c} rises (2), subjects with higher HbA_{1c} variability would "accumulate" a surplus of risk

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

	Nonadvanced retinopathy		Advanced retinopathy	
Variables	OR (95% CI)	*P*	OR (95% CI)	*P*
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				0.084
Never			1.0	
Former			0.904 (0.748–1.093)	0.297
Current			0.753 (0.583–0.972)	0.030
Hypertension	1.317 (1.074–1.615)	0.008	2.541 (1.815–3.557)	0.0001
Previous CVD event	1.319 (1.146–1.517)	0.0001	1.288 (1.073–1.546)	0.007
Diabetes treatment		0.0001		0.0001
Diet	1.0		1.0	
OHA	2.084 (1.519–2.860)	0.0001	1.887 (1.187–3.001)	0.007
OHA + insulin	4.113 (2.859–5.918)	0.0001	7.516 (4.574–12.350)	0.0001
Insulin	3.089 (2.185–4.365)	0.0001	5.529 (3.420–9.938)	0.0001
Albuminuria		0.0001		0.0001
Normoalbuminuria	1.0		1.0	
Microalbuminuria	1.400 (1.206–1.625)	0.0001	1.854 (1.535–2.239)	0.0001
Macroalbuminuria	1.753 (1.324–2.320)	0.0001	3.128 (2.317–4.224)	0.0001
eGFR				0.0001
≥90 mL/min/1.73 m^2			1.0	
60–89 mL/min/1.73 m^2			1.256 (1.007–1.567)	0.43
30–59 mL/min/1.73 m^2			1.734 (1.319–2.280)	0.0001
<30 mL/min/1.73 m^2			3.531 (2.132–5.847)	0.0001
Model 1				
HbA_{1c}-MEAN, 1% increment	1.236 (1.167–1.308)	0.0001	1.263 (1.178–1.354)	0.0001
Model 2				
HbA_{1c}-MEAN, 1% increment	1.326 (1.245–1.412)	0.0001	1.263 (1.178–1.354)	0.0001
HbA_{1c}-SD 1% increment	0.917 (0.758–1.110)	0.093		
Model 3				
HbA_{1c}-MEAN quartiles		0.0001		0.0001
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
HbA_{1c}-SD quartiles				

ORs of variables except HbA_{1c}-MEAN and HbA_{1c}-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.

in the periods spent at the upper end of their HbA_{1c} range. This hypothesis might be indirectly supported by our observation that the effect of HbA_{1c} variability is a statistically significant effect in the higher quartile of HbA_{1c}-SD.

Finally, the link between fluctuations of HbA_{1c} and risk of microvascular complications might relate to the fact that patients with a higher HbA_{1c}-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 HbA_{1c}-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 HbA_{1c} 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) HbA_{1c} 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 HbA_{1c} measures, independently of their HbA_{1c} variability, although a selection bias cannot be ruled out conclusively.

Other possible limitations concerning HbA_{1c} values are that they were performed in each center as a part of the patient's standard care, with no prespecified

intervals between HbA_{1c} measurements, and that the number of measures per individual varied from 3 to 5. However, noncentralized measurements did not affect intraindividual variability, intervals between measurements ranged from 6 to 9 months, and adj-HbA_{1c}-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 (i.e., 4–5) HbA_{1c} measurements (12) or a series of 2-year HbA_{1c} values (13), as in our study, did not change the results. Finally, potential limitations concerning noncentralized assessment of DN and DR have been addressed in previous RIACE reports (14,17,20).

In conclusion, in patients with type 2 diabetes, HbA_{1c} variability affects albuminuria and albuminuric CKD phenotypes independently of (or instead of) average HbA_{1c}, even after adjustment for known risk factors for microvascular complications. On the contrary, HbA_{1c} variability has no effects on DR, which is mainly dependent on HbA_{1c}.

Acknowledgments—This work was supported by the Research Foundation of the Italian Society of Diabetology (Fo.Ri.SID) and the Diabetes, Endocrinology, and Metabolism (DEM) Foundation, and by unconditional grants from Eli-Lilly, Takeda, Chiesi Farmaceutici, and Boehringer Ingelheim. The sponsors had no role in design and conduct of the study; collection, management, and interpretation of the data; or in the preparation, review, and approval of the manuscript. No other potential conflicts of interest relevant to this article were reported.

G.Pe. and G.Pu. researched data and wrote the manuscript. A.S. and A.N. researched data and reviewed and edited the manuscript. E.B., C.F., E.O., G.Z., S.M., F.C., O.L., and L.L. researched data and contributed to discussion. G.Pu. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

The authors thank the RIACE Investigators for participating in this study (the complete list is available in the Supplementary Data).

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