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Activation of Oxidative Stress by Acute Glucose Fluctuations Compared With Sustained Chronic Hyperglycemia in Patients With Type 2 Diabetes

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DIABETES IS CHARACTERIZED BY development of specific microvascular complications and by a high incidence of accelerated atherosclerosis.¹⁻⁴ Even though a large number of studies have investigated and compared the roles of the different factors that participate in diabetic vascular complications, an accurate assessment of their respective contributions is still difficult.^{2,3,6} However, as demonstrated by many trials, microvascular and macrovascular complications are mainly^{7,8} or partly^{4,8} dependent on hyperglycemia.

At least 4 major pathways are involved in hyperglycemia-induced vascular damages: (1) enhanced polyol activity, causing sorbitol and fructose accumulation; (2) increased formation of advanced glycation end products; (3) activation of protein kinase C and nuclear factor κ B; and (4) increased hexosamine pathway flux.⁹ There are many reasons to think that hyperglycemic states trigger all of these deleterious metabolic events through a single process:

See also pp 1688 and 1707.

Context Glycemic disorders, one of the main risk factors for cardiovascular disease, are associated with activation of oxidative stress.

Objective To assess the respective contributions of sustained chronic hyperglycemia and of acute glucose fluctuations to oxidative stress in type 2 diabetes.

Design, Setting, and Participants Case-control study of 21 patients with type 2 diabetes (studied 2003-2005) compared with 21 age- and sex-matched controls (studied in 2001) in Montpellier, France.

Main Outcome Measures Oxidative stress, estimated from 24-hour urinary excretion rates of free 8-iso prostaglandin $F_{2\alpha}$ (8-iso $PGF_{2\alpha}$). Assessment of glucose fluctuations was obtained from continuous glucose monitoring system data by calculating the mean amplitude of glycemic excursions (MAGE). Postprandial contribution to glycemic instability was assessed by determining the postprandial increment of glucose level above preprandial values (mean postprandial incremental area under the curve [AUCpp]). Long-term exposure to glucose was estimated from hemoglobin A_{1c} , from fasting glucose levels, and from mean glucose concentrations over a 24-hour period.

Results Mean (SD) urinary 8-iso $PGF_{2\alpha}$ excretion rates were higher in the 21 patients with diabetes (482 [206] pg/mg of creatinine) compared with controls (275 [85] pg/mg of creatinine). In univariate analysis, only MAGE ($r=0.86$; $P<.001$) and AUCpp ($r=0.55$; $P=.009$) showed significant correlations with urinary 8-iso $PGF_{2\alpha}$ excretion rates. Relationships between 8-iso $PGF_{2\alpha}$ excretion rates and either MAGE or AUCpp remained significant after adjustment for the other markers of diabetic control in multiple linear regression analysis (multiple $R^2=0.72$ for the model including MAGE and multiple $R^2=0.41$ for the model including AUCpp). Standardized regression coefficients were 0.830 ($P<.001$) for MAGE and 0.700 ($P=.003$) for AUCpp.

Conclusions Glucose fluctuations during postprandial periods and, more generally, during glucose swings exhibited a more specific triggering effect on oxidative stress than chronic sustained hyperglycemia. The present data suggest that interventional trials in type 2 diabetes should target not only hemoglobin A_{1c} and mean glucose concentrations but also acute glucose swings.

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overproduction of superoxide by the mitochondrial electron-transport chain. This elegant and unifying theory developed by Brownlee¹⁰ seems to indicate that activation of the oxidative stress by hyperglycemia plays a major role in the pathogenesis of diabetic complications.

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Because all these metabolic alterations occur more particularly in endothelial cells, it has been postulated that they can result in endothelial dysfunction and contribute to vascular damages.^{9,10}

From a more practical point of view, exposure to glycemic disorders can be described as a function of 2 components: the duration and magnitude of chronic sustained hyperglycemia and the acute fluctuations of glucose over a daily period.^{4,8,11-13} The first component was integrated by hemoglobin (Hb) A_{1c}, which depends on both interprandial and postprandial hyperglycemia, the percentage of each contributor being modulated by the degree of diabetic con-

trol.¹⁴ The second component, ie, the acute fluctuations of glucose around a mean value, is more difficult to assess, but the recent development of devices that permit continuous glucose monitoring on an ambulatory basis certainly represents a new approach for studying the influence of acute blood glucose fluctuations in real life.¹⁵⁻¹⁷ By applying this technology, we have attempted to gain further insight into the respective role of both sustained chronic hyperglycemia and acute glucose fluctuations on activation of oxidative stress as estimated from measurement of daily urinary excretion of the F₂ isoprostane 8-iso prostaglandin F_{2α} (8-iso PGF_{2α}),

which is considered a well-recognized marker of oxidative stress.^{18,19}

METHODS

Patients

Twenty-one patients with type 2 diabetes in Montpellier, France, were included from 2003-2005. The patients were entered consecutively without any selection based on HbA_{1c} levels (range, 7.5%-12.5%; mean [SD], 9.6% [1.3%]). Eligibility for the study was based on a diagnosis over a minimum 6-month period. Main clinical and laboratory characteristics of the patients are given in TABLE 1. Nineteen patients were treated with oral antidiabetic agents: glyburide alone (10-15 mg/d in 3 patients) or a combination of metformin (1700 mg/d) and glyburide (15 mg/d) (in 16 patients). All patients except 2 were participating in a constant drug regimen and all followed a stable weight-controlling diet for at least 3 months before enrolling in the study. Exclusion criteria included patients treated with steroid or nonsteroidal anti-inflammatory drugs, patients who had experienced an acute concurrent illness during the 3-month period preceding the investigation, and all individuals with a glomerular filtration rate of less than 60 mL/min per 1.73 m² using the Cockcroft-Gault formula.²⁰ The study was conducted after each patient had given oral informed consent, in accordance with European directives that require no approval from an ethics committee for the study design as described herein.²¹

Control data were retrospectively drawn from a vitamin E study in healthy individuals in 2001 at the University of Montpellier.

Study Protocol

Subcutaneous interstitial glucose levels were monitored on an ambulatory basis over a period of 3 consecutive days by using the second-generation Minimed continuous glucose monitoring system (Medtronic, Northridge, Calif).^{15,16} The sensor was inserted on day 0 and removed on day 3 at mid morning. The data were downloaded to a computer for

Table 1. Clinical and Laboratory Characteristics*

Characteristics	Patients With Type 2 Diabetes (n = 21)	Age- and Sex-Matched Controls (n = 21)
Age, y	63.6 (7.6)	64.5 (6.2)
No. of men/women	13/8	13/8
BMI	30.5 (3.8)	23.3 (2.9)
Blood pressure, mm Hg		
Systolic	133 (32)	130 (15)
Diastolic	71 (16)	77 (9)
Diabetes treatment, No. (%)		
Diet alone	2 (10)	0
Sulfonylurea alone	3 (14)	0
Metformin alone	0	0
Combination (sulfonylurea + metformin)	16 (76)	0
Other treatments, No. (%)		
Lipid-lowering drugs	5 (24)	0
Antihypertensive drugs	7 (33)	0
Hemoglobin A _{1c} , %	9.6 (1.3)	ND
Total cholesterol, mg/dL	205 (31)	216 (23)
Triglycerides, mg/dL	168 (88)	98 (62)
HDL cholesterol, mg/dL	54 (39)	60 (16)
LDL cholesterol, mg/dL	108 (39)	132 (23)
Free fatty acids, μmol/dL	1.36 (0.46)	ND
Plasma creatinine, mg/dL	1.0 (0.3)	0.8 (0.2)
Glucose, mg/dL		
24-Hour†	190 (65)	ND
Fasting plasma	209 (34)	82 (9)
Fasting plasma insulin, μU/mL	9 (7)	ND
8-Iso prostaglandin F _{2α} excretion rate, pg/mg of creatinine	482 (206)	275 (85)
MAGE, mg/dL of glucose	75 (25)	ND
AUC _{pp} , mg/dL • h of glucose	407 (316)	ND

Abbreviations: AUC_{pp}, postprandial incremental area under the curve; BMI, body mass index, calculated as weight in kilograms divided by the square of height in meters; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MAGE, mean amplitude of glycemic excursions; ND, not determined.

SI conversions: To convert total, HDL, and LDL cholesterol to mmol/L, multiply by 0.0259; to convert triglycerides to mmol/L, multiply by 0.0113; to convert creatinine to μmol/L, multiply by 88.4; to convert glucose to mmol/L, multiply by 0.0555.

*Data are expressed as mean (SD) unless otherwise specified.

†From continuous glucose monitoring system measurements.

evaluation of glucose variations, but calculations of glucose variations were limited to data obtained on days 1 and 2 to avoid bias due to both insertion and removal of the sensor and, thus, to insufficient stabilization of the monitoring system. The characteristic glucose pattern of each patient was calculated by averaging the profiles obtained on study days 1 and 2.

On day 1, 24-hour urinary samples were collected. Urinary samples were stored at -80°C until analyses for 8-iso $\text{PGF}_{2\alpha}$ and creatinine were carried out. On day 0, after an overnight fast, at breakfast time and before the sensor insertion, venous blood samples were drawn into tubes containing EDTA. Plasma was separated from the cells within 1 hour after withdrawal, and the samples thus obtained were further processed for the following laboratory measurements: creatinine, glucose, HbA_{1c} , total cholesterol, high-density lipoprotein cholesterol, triglycerides, insulin, and free fatty acids. Systolic and diastolic blood pressure were determined using the right arm of patients after at least 5 minutes of resting in a supine position. The average of 3 measurements was used for analysis.

Laboratory Measurements

Glucose concentrations in plasma were measured by a hexokinase method using a Synchron CX4/CX5 glucose analyzer (Beckman Instruments, Fullerton, Calif). Both the intra-assay and interassay coefficients of variation were 2% or less at values of less than 126 mg/dL (7 mmol/L). Fasting plasma insulin was measured by using a specific insulin assay (bi-insulin immunoradiometric assay, Schering CIS Bio International, Gif sur Yvette, France). The intra-assay and interassay coefficients of variation varied respectively from 3.8% to 2.6% and from 8% to 2.6% at values between 8 mU/L and 130 mU/L. Hemoglobin A_{1c} was measured using a high-performance liquid chromatography assay (Menarini Diagnostics, Florence, Italy). The intra-assay and interassay coefficients of variation were less than 3% at values less than 8%.

Total cholesterol was determined on a PRO analyzer (Konelab, Thermo Elec-

tron Corp, Vantaa, Finland) by a cholesterol oxidase method (kit from Thermo Electron Corp). Triglycerides were measured on the same analyzer by an enzymatic method using an automated glycerol blank (kit from Thermo Electron Corp). High-density lipoprotein cholesterol was quantified without any pretreatment using polyethylene glycol-modified enzymes and dextran sulfate (kit from Thermo Electron Corp). Low-density lipoprotein cholesterol concentrations were calculated from measurements of the concentrations of total cholesterol, triglycerides, and high-density lipoprotein cholesterol using the formula of Friedewald et al.²² Serum free fatty acids were measured by the acidimetric method of Dole.²³

Plasma and urinary creatinine levels were determined by using an enzymatic method (Randox Laboratories, Crumlin, Wales) and the Jaffe kinetic method for plasma and urine, respectively.

Free 8-iso $\text{PGF}_{2\alpha}$ is the most frequently measured F_2 isoprostane in body fluids. In the present study, this isomer was measured using an enzyme immunoassay method (Cayman Chemical SPI-BIO, Montigny-le-Brettonneux, France). Purification and extraction of urine samples were performed before assay. The pH of urine (1 mL) was adjusted to 2.2 with hydrochloride, diluted in water to 10 mL, and added to 3H-8-iso $\text{PGF}_{2\alpha}$ as an internal standard. The aliquots were then treated with 2 extraction procedures, successively using an inverse-phase and an aminopropyl-phase cartridge (Seppak VAC RC C-18 and VAC RC NH2, Waters SA, Guyancourt, France) as previously described.²⁴ The extracted samples were assayed by using the 8-isoprostane enzyme immunoassay method with the following main crossed reactions: 8-iso PGF_3 (21%) and 8-iso prostaglandin E_2 (1.8%). The intra-assay and interassay coefficients of variation were 3% and 3.4%. Isoprostane concentrations are expressed as picograms per milligram of urinary creatinine.

As in most studies, we have used determinations in urinary samples to avoid the ex vivo artifactual formation of isoprostanes resulting from auto-

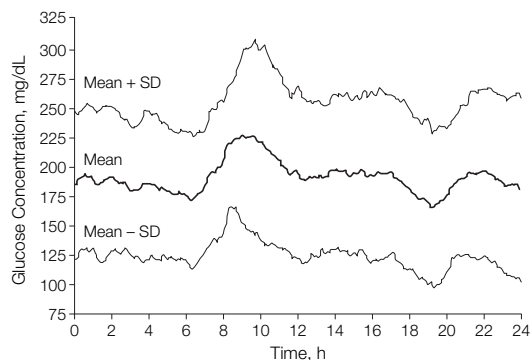
oxidation of lipids. In the present study, measurement was made only by the immunoassay method. The concurrent assay using gas chromatography-mass spectrometry was not performed. In several studies, both methods have been used.²⁴ When immunoassay and gas chromatography were concomitantly performed, the results did not necessarily correlate as an identity line, indicating that the 2 methods do not measure the same compounds.²⁵ However, it is generally acknowledged that both methods provide more reliable assessments of lipid peroxidation than any other test in healthy individuals and in those with different types of diseases, including diabetes.²⁶

Assessment of Glycemic Instability

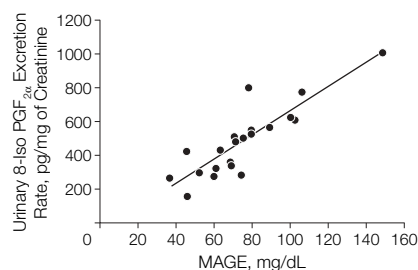
The mean amplitude of glycemic excursions (MAGE), which has been described by Service et al,²⁷ was used in the present study for assessing glucose fluctuations during the day. For this purpose, we used the glucose profiles obtained from continuous glucose monitoring system data on study days 1 and 2; ie, from continuous monitoring for 48 hours. This parameter was designed to quantify major swings of glycemia and to exclude minor ones. For this reason, only increases of more than 1 SD of the mean glycemic values were taken into account. Calculation of the MAGE was obtained by measuring the arithmetic mean of the differences between consecutive peaks and nadirs; measurement in the peak-to-nadir or nadir-to-peak direction was determined by the first qualifying excursion. The measurement of this parameter, which has been proved independent of mean glycemia, is of particular interest since the greater the MAGE, the higher the glycemic instability.²⁸

Assessment of Postprandial Glucose Excursions

The glucose patterns obtained from each patient on study days 1 and 2 were submitted to the following readings and calculations: first, the 3 preprandial values (before breakfast, lunch, and dinner) were read on the time curves. Sec-

Figure 1. 24-Hour Recordings From the Continuous Glucose Monitoring System in 21 Patients

To convert glucose to mmol/L, multiply values by 0.0555.

Figure 2. Linear Correlation Between 24-Hour Urinary Excretion Rates of 8-Iso Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and Mean Amplitude of Glycemic Excursions (MAGE)

$r=0.86$; $P<.001$.

ond, the incremental areas above preprandial glucose values were calculated over a 4-hour period following the beginning of each meal. Third, the 3 incremental areas obtained on each study day were summed and averaged on study days 1 and 2 to calculate the mean postprandial incremental area under the curve (AUCpp) in each patient.

Statistical Analysis

Mean comparisons were made using the *t* test for unpaired data. Simple linear correlations were calculated using the concept of least squares and by determining the Pearson correlation coefficient *r*. Multiple regression models were used to explore the influence of different variables on urinary 8-iso $PGF_{2\alpha}$ excretion rates and to adjust for covariates.²⁹ We calculated the number of patients required for the study to reject the null hy-

pothesis 99% of the time (ie, with a 1-tailed type II error rate of .01) when *r* was 0.80 or higher with a 2-tailed type I error at the .05 level of significance. Because this calculation led to a sample size of at least 19, the number of required patients was set at 21.²⁹ Analyses were performed with the STATVIEW statistical package, version 5 for Macintosh (SAS Institute Inc, Cary, NC).

RESULTS

The mean glucose patterns obtained from the continuous glucose monitoring system in the 21 patients are shown in FIGURE 1 (mean [SD] glucose concentration over 24 hours, 190 [65] mg/dL).

Comparison of Urinary Excretion Rates of 8-Iso $PGF_{2\alpha}$

The mean urinary excretion rate of 8-iso $PGF_{2\alpha}$ was significantly higher ($P<.001$) in the 21 patients with type 2 diabetes (482 [SD, 206] pg/mg of creatinine) than in 21 age- and sex-matched controls (275 [SD, 85] pg/mg of creatinine). The clinical and laboratory characteristics of the 21 controls are given in Table 1.

Relationships Between 8-Iso $PGF_{2\alpha}$ Excretion and Markers of Diabetic Control

Univariate Analysis. When urinary excretion rates of 8-iso $PGF_{2\alpha}$ were tested for simple linear correlations against markers of glucose control, the strongest

correlation was found with MAGE ($r=0.86$; $P<.001$) (FIGURE 2). A statistically significant correlation was also observed with AUCpp ($r=0.55$; $P=.009$). In contrast, urinary excretion rates of 8-iso $PGF_{2\alpha}$ did not correlate with fasting plasma insulin or with any of the parameters that are usually considered markers of mean glucose exposure, including HbA_{1c} , fasting plasma glucose, and mean daily glucose concentrations (the latter being calculated from the continuous glucose monitoring system) (TABLE 2). However, it should be noted that fasting plasma glucose correlated with 8-iso $PGF_{2\alpha}$ excretion rates when patients with type 2 diabetes were analyzed in a pooled manner with controls ($r=0.62$; $n=42$).

Multivariate Analysis. Multiple linear regression analyses were performed to assess the independent effects of markers of diabetic control on urinary 8-iso $PGF_{2\alpha}$ excretion rates (TABLE 3). These markers included mean glucose concentrations over 24 hours, fasting plasma insulin, AUCpp, and MAGE. Because a strong intercorrelation was observed between MAGE and AUCpp ($r=0.728$; ie, $R^2=0.53$) in the univariate analysis (Table 2), 2 independent models were tested, 1 including the MAGE (model 1) and 1 the AUCpp (model 2). The AUCpp substitution for MAGE resulted in a decrease in a multiple R^2 (coefficient of determination) from 0.72 to 0.41; ie, a decrease of 43% (Table 3).

Relationships Between 8-Iso $PGF_{2\alpha}$ Excretion and Nonglycemic Clinical and Laboratory Variables

In the univariate analysis, no relationships were observed when urinary 8-iso $PGF_{2\alpha}$ was tested for simple correlation with body mass index ($r=0.14$), systolic ($r=-0.10$) or diastolic ($r=-0.08$) blood pressure, and lipid concentrations (total cholesterol, triglycerides, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, and free fatty acids) (Table 2). This absence of relationships was confirmed by the fact that nonglycemic variables failed to enter the

Table 2. Pearson Correlation Coefficients Between Urinary Excretion Rates of 8-Iso PGF_{2α} and Markers of Diabetic Control and Nonglycemic Metabolic Variables

	Fasting Plasma Glucose Level	Mean Glucose Level*	HbA _{1c}	Fasting Plasma Insulin Level	MAGE	AUCpp	TC	HDL-C	LDL-C	Triglycerides	FFAs
Mean glucose level*	0.570†										
HbA _{1c}	0.225	0.445†									
Fasting plasma insulin	0.106	0.196	0.269								
MAGE	0.435†	0.246	-0.081	0.288							
AUCpp	0.440†	0.521†	-0.049	-0.065	0.728†						
TC	-0.038	0.216	0.213	-0.195	-0.164	0.206					
HDL-C	-0.097	-0.184	-0.041	-0.285	-0.038	-0.172	-0.341				
LDL-C	-0.099	-0.017	0.317	-0.214	-0.074	0.149	0.719†	-0.344			
Triglycerides	0.166	0.354	0.257	0.198	0.133	0.290	0.426	-0.536†	0.124		
FFAs	-0.173	0.041	-0.332	0.018	0.167	0.145	-0.043	0.054	-0.370	0.367	
8-Iso PGF _{2α}	0.410	0.218	0.061	0.365	0.863†	0.547†	-0.008	-0.113	0.006	0.226	0.195

Abbreviations: AUCpp, postprandial incremental area under the curve; FFA, free fatty acid; Hb, hemoglobin; HDL, high-density lipoprotein; 8-iso PGF_{2α}, 8-iso prostaglandin F_{2α} excretion rate; LDL, low-density lipoprotein; MAGE, mean amplitude of glycemic excursions; TC, total cholesterol.

*Mean glucose concentrations were calculated over 24 hours.

†P<.05.

model when the stepwise regression analysis was applied to both glycemic and nonglycemic parameters.

Relationship Between MAGE and Therapy for Diabetes

Patients were classified into 2 groups according to whether they received a 2-drug treatment (n=16) or another treatment (ie, a 1-drug treatment or diet alone). MAGE medians were not significantly different (74.5 mg/dL [4.13 mmol/L] in the first group and 68.0 mg/dL [3.77 mmol/L] in the second group) by using nonparametric statistics (Mann-Whitney test), indicating that there was no relationship between MAGE and therapy for diabetes.

COMMENT

The results of the present study show that the production of 8-iso PGF_{2α} is higher in patients with type 2 diabetes than in healthy controls. Furthermore, acute glucose fluctuations were strongly correlated with urinary excretion of 8-iso PGF_{2α}, while no relationship was observed when urinary 8-iso PGF_{2α} excretion rates were plotted against main markers of sustained hyperglycemia (HbA_{1c} and mean daily glucose concentrations). In our patients, the mean glucose concentration (190 mg/dL [10.5 mmol/L]), as obtained from the continuous glucose monitoring system, is not comparable with the

Table 3. Independent Effects of Markers of Diabetic Control on Urinary 8-Iso PGF_{2α} Excretion Rates

	Standardized Regression Coefficient	t	P Value	Adjusted R ² of the Model
Model 1			<.001	0.72
Mean glucose concentrations*	-0.012	-0.093	.93	
MAGE	0.830	6.551	<.001	
Fasting plasma insulin	0.128	1.020	.32	
Model 2			.007	0.41
Mean glucose concentrations*	-0.236	-1.128	.27	
AUCpp	0.700	3.405	.003	
Fasting plasma insulin	0.456	2.550	.02	

Abbreviations: AUCpp, postprandial incremental area under the curve; 8-iso PGF_{2α}, 8-iso prostaglandin F_{2α} excretion rate; MAGE, mean amplitude of glycemic excursions.

*Mean glucose concentrations were calculated over 24 hours.

mean plasma glucose concentration (261 mg/dL [14.5 mmol/L]) that should normally be expected from the mean HbA_{1c} level of 9.6%.³⁰ This apparent inconsistency is partly due to the fact that the monitoring system underestimated the real glucose value as compared with the reference method, since relative absolute differences of 12.8% to 15.7% have been reported when continuous glucose monitoring system values were compared with concurrent laboratory plasma glucose analysis.³¹ In the present study, we found an absolute difference of 19 mg/dL (1.05 mmol/L) between the monitoring system value and plasma glucose concentrations measured before breakfast. Another explanation could be that patients were better adherent to lifestyle rec-

ommendations when wearing the monitoring system.

Because isoprostanes are collectively formed from free radical-mediated oxidation of arachidonic acid,¹⁸ the measurement of specific isoprostane isomers has been proposed to be a good indicator of oxidative stress,¹⁹ a metabolic disorder that has been described as one of the main causes of vascular disease.^{9,10,32} In the present study, the measurement of 8-iso PGF_{2α} in 24-hour urinary samples was based on several arguments. The first is that arachidonic acid, the metabolic precursor of isoprostanes, is ubiquitously distributed in cell membranes; therefore, measurements of urinary isoprostanes most likely provide an excellent reflection of the activation of oxidative stress in the whole

body, even though it seems likely that a significant but largely undetermined proportion of urinary isoprostanes is directly generated by the endothelial and mesangial cells of the glomerulus.³³

The second reason for choosing urinary rather than plasma samples was based on the fact that most of the arachidonic acid derivatives, including 8-iso PGF_{2α}, have a short plasma half-life.³⁴ Therefore, urinary determinations probably provide a more reliable estimation of the 24-hour fluctuations in isoprostane production than blood sampling at fixed time points. At present, many studies have investigated the relationship between isoprostane production and several risk factors that are well-recognized major contributors to cardiovascular diseases.³⁵ For instance, in vivo formation of 8-iso PGF_{2α} is increased in patients with either type 1 or type 2 diabetes.³⁶⁻³⁸ As reported by Davi et al,³⁶ improvements in diabetes control seem to result in reduction of urinary excretion rates of 8-iso PGF_{2α}. Other risk factors of atherosclerosis such as hypertension,³⁹ regular smoking,⁴⁰ hyperlipidemia,⁴¹ and obesity⁴² have also been described as being associated with elevated urinary excretion rates of isoprostanes. However, we found no correlation between urinary 8-iso PGF_{2α} excretion rates and such parameters as body mass index, blood pressure, and serum lipid concentrations.

Another point of the discussion concerns a possible specific effect of oral antidiabetic drugs⁴³ and statins⁴⁴ on oxidative stress. At present, it has not been established whether the influence of the aforementioned treatments on oxidative stress are more dependent on their glucose- or lipid-lowering effects than on a specific antioxidative action.^{36,45} In patients with type 2 diabetes, many studies have clearly established that complications are mainly due to chronic hyperglycemia that exerts its deleterious effects through several mechanisms: accelerated glycation of cellular and extracellular proteins,^{9,46} diabetic dyslipidemia,⁴⁷ platelet activation, impaired fibrinolysis,⁴⁸ and altered endothelial metabolism.⁹ Even though the effects of treatments aimed at reducing oxidative

damage to proteins and lipids in patients with increased risk factors of atherothrombosis remain controversial,^{45,49} it has been proposed that activation of oxidative stress plays a pivotal role in the effects of the above-mentioned mechanisms and risk factors¹⁰ associated with, or induced by, sustained hyperglycemia.

However, there is increasing evidence that other glycemic disorders such as rapid glucose swings might play an important role.¹¹ For instance, emphasis has been recently given to the relationship between postprandial hyperglycemia and, more generally, hyperglycemic spikes and diabetic complications.⁵⁰ By using different methods, including epidemiological,¹² interventional,⁵¹ or pathophysiological studies,⁵² several authors have demonstrated that postprandial hyperglycemia is certainly an independent risk factor of vascular complications in type 2 diabetes. For instance, it has been established that postprandial hyperglycemia induces an overproduction of superoxide which, after reacting with nitric oxide, produces a subsequent nitrosative stress with generation of such metabolic derivatives as peroxynitrite and nitrotyrosine.⁵⁰ The toxicity of these substances can lead to endothelial damages and, furthermore, to microvascular and macrovascular complications. By reducing postprandial excursions, it has been demonstrated that oxidative and nitrosative stress can be diminished.⁵²

Glucose variations over time are not limited to postprandial hyperglycemic excursions since blood glucose concentrations in patients with diabetes are always fluctuating from hyperglycemic peaks to glucose nadirs. Such fluctuations are particularly marked in type 1 diabetes and, to a lesser degree, in patients with type 2 diabetes treated with insulin. However, non-insulin-dependent patients can also experience such peak-and-trough patterns with acute glucose variations. Peaks are usually corresponding to maximum values after meals, particularly at mid morning,⁵³ while troughs are observed over interprandial periods,⁵⁴ especially in patients who are treated with insulin secretagogues and

who are at risk of hypoglycemic episodes.⁵⁵

The multivariate analysis showed that the coefficients of determination (R^2) between urinary 8-iso PGF_{2α} and markers of glycemic control dropped from 0.72 to 0.41, ie, by 43% after AUCpp substitution for MAGE. Since the glycemic fluctuations as estimated from MAGE indexes reflect both upward and downward glucose changes, whereas AUCpp values are only markers of upward variations, there is some reason to think that MAGE indexes are wider integrators of glycemic variations than the AUCpp. By returning to the R^2 value (0.53) of the univariate analysis between MAGE and AUCpp (considered the y-axis and x-axis values, respectively) and by further calculating the coefficient of non-determination ($1 - R^2 = 0.47$),²⁹ it appears that the proportion of total variation of MAGE not attributable to the dependence on AUCpp was 47%, a percentage comparable with that (43%) found for the decrement in R^2 after AUCpp substitution for MAGE in the multiple regression analysis, with urinary 8-iso PGF_{2α} as the dependent variable. These observations provide a possible explanation for the differences observed between the influences of MAGE and AUCpp. We therefore suggest that the triggering effect of acute glycemic excursions on oxidative stress should be integrated into glycemic disorders that are larger than acute postmeal spikes,^{39,51,53} ie, into rapid glucose swings including the declines from relatively high glucose levels during postprandial periods⁵³ to low values or even to asymptomatic hypoglycemia, as observed over interprandial periods.^{54,55} As a consequence, low glycemic levels in type 2 diabetes might stimulate oxidative stress; this observation is consistent with the fact that platelet aggregation can be activated during moderate insulin-induced hypoglycemia in type 1 diabetes.⁵⁶

In conclusion, the present study demonstrates a significant relationship between acute glucose swings and activation of oxidative stress. In contrast, no relationship was observed with any parameters of long-term glucose expo-

sure. A weakness in the present study is its cross-sectional, observational nature, and it is therefore difficult to draw causal relationships. However, because activation of oxidative stress is one of the main mechanisms that leads to diabetic complications,¹⁰ the present data open the field to conduct interventional studies aimed at treating glycemic disorders not only by reducing HbA_{1c} and mean hyperglycemia but also by flattening acute glucose fluctuations.

Author Contributions: Dr Monnier had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Monnier, Colette.

Acquisition of data: Mas, Ginet, Michel, Villon, Colette.
Analysis and interpretation of data: Monnier, Cristol, Colette.

Drafting of the manuscript: Monnier.

Critical revision of the manuscript for important intellectual content: Mas, Ginet, Michel, Villon, Cristol, Colette.

Statistical analysis: Monnier, Colette.

Study supervision: Monnier.

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