Glycemic Variability

Should we and can we prevent it?

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Diabetes is characterized by glycemic disorders that include both sustained chronic hyperglycemia and acute glucose fluctuations. There is now cogent evidence for the deleterious effects of sustained chronic hyperglycemia that results in excessive protein glycation and generation of oxidative stress. The role of glucose variability from peaks to nadirs is less documented, but there are many reasons to think that both upward (postprandial) and downward (interprandial) acute fluctuations of glucose around a mean value activate the oxidative stress. As a consequence, it is strongly suggested that a global antidiabetic strategy should be aimed at reducing to a minimum the different components of dysglycemia (i.e., A1C, fasting and postprandial glucose, as well as glucose variability). All the therapeutic agents that act on postprandial glucose excursions seem of particular interest for reducing the latter parameter (i.e., the glucose instability). Particular attention should be paid to such emerging therapeutic agents as the glucagon-like peptide 1 agonists and the dipeptidyl peptidase (DPP)-IV inhibitors that act through the incretin pathway.

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Diabetes is characterized by the development of specific microvascular complications and a high incidence of accelerated atherosclerosis (1–4). Although a large number of studies have investigated and compared the roles of the many factors involved in diabetic vascular complications, an accurate assessment of their respective contributions is still difficult (2,5,6). However, as demonstrated by many trials, microvascular and macrovascular complications are mainly (7,8) or partly (4,8) dependent on dysglycemia, which has two components: chronic sustained hyperglycemia and acute glycemic fluctuations from peaks to nadirs. Both components lead to diabetes complications through two main mechanisms: excessive protein glycation and activation of oxidative stress. A few years ago, these two mechanisms were unified in an elegant theory that suggested that the glycemic disorders observed in diabetic patients result in an activation of oxidative stress with an overproduction of superoxide by the mitochondrial electron-transfer chain (9,10). This activation in turn produces a cascade of such deleterious metabolic events as enhanced polyol activity, increased formation of advanced glycation end products, activation of protein kinase C and nuclear factor κB, and increased hexosamine pathway flux (9,10). It is now well established that hyperglycemia both at fasting and during postprandial periods results in exaggerated and accelerated glycation. For instance, all the studies conducted in type 1 and type 2 diabetes have clearly shown a strong positive relationship between A1C levels and plasma glucose levels at fasting and over postprandial periods (11–13), with the strongest correlation being observed between A1C and mean plasma glucose levels. The latter relationship was considered sufficiently demonstrative to serve as a reference in the recent standards of medical care in diabetes that are published every year by the American Diabetes Association (14,15). A1C is first recognized as a reliable marker for the overall glucose exposure and its direct consequence, an excessive rate of glycation (16,17). Second, A1C is an integrator of both fasting and postprandial glycemic disorders. As a consequence, it is not surprising that fasting and postprandial hyperglycemia were identified separately or concomitantly as major risk factors for diabetes complications. The U.K. Prospective Diabetes Study demonstrated that reductions in A1C and fasting blood glucose levels were accompanied by substantial decreases in the risk of all diabetes-related end points, particularly microvascular complications (18). Two years later, the authors of that study reported that the risks for myocardial infarction and microvascular complications were diminished by 14 and 37%, respectively, for each 1% reduction in A1C (4). In 1996, Hanefeld et al. (19) demonstrated in the Diabetes Intervention Study that postprandial hyperglycemia was a better predictor of subsequent myocardial infarction and mortality than fasting hyperglycemia. Further landmark studies have confirmed this result, suggesting that postprandial hyperglycemia is an independent risk factor for macrovascular diseases (20,21). However, besides the impact of fasting and postprandial glucose on the glycation process, several other mechanisms may be involved in diabetes complications. For instance, one question that should be raised is, "Should we prevent glucose variability?" To respond to this question, the first part of this review article is mainly devoted to the analysis of the contributions of fasting plasma glucose, postprandial glucose, and acute glucose fluctuations to the activation of oxidative stress and thus to the respective impacts of these glycemic disorders on diabetes complications.

Several markers have been used to assess oxidative stress and the antioxidant status in patients with diabetes (22). The short plasma half-life of these markers is one of the limiting factors for the assessment of oxidative stress in plasma samples. Thus, when available, urinary determinations provide a more reliable estimation of the activation of oxidative stress than plasma measurements (23,24). Accordingly, the determination of such specific isoprostane isomers as

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Abbreviations: 8-iso-$PGF_{2\alpha}$, 8-iso-prostaglandin $F_{2\alpha}$; MAGE, mean amplitude of glycemic excursions.

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8-iso-prostaglandin $F_{2\alpha}$ (8-iso-$PGF_{2\alpha}$) in urine has been proposed. Isoprostanes are collectively formed from free radical–mediated oxidation of arachidonic acid (25). Because this fatty acid is ubiquitously distributed in cell membranes, measurements of urinary isoprostanes most likely provide an excellent reflection of the activation of oxidative stress in the whole body. In the different studies that have been conducted in diabetes, plasma and urinary metabolites have been alternatively or simultaneously used as oxidative stress markers. The current available data are analyzed in the next section.

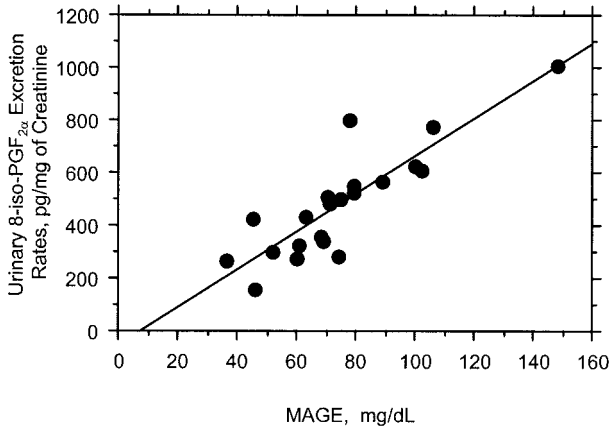

Figure 1—*Linear correlation between 24-h urinary excretion rates of 8-iso-$PGF_{2\alpha}$ and MAGE in 21 patients with type 2 diabetes (28, printed with permission from the* Journal of the American Medical Association*).*

GLUCOSE VARIABILITY: SHOULD WE PREVENT IT?

Contribution of fasting plasma glucose to the activation of oxidative stress

From several studies, there is cogent evidence that hyperglycemia is associated with increased formation and urinary excretion rate of 8-iso-$PGF_{2\alpha}$ (26). The urinary excretion rate of 8-iso-$PGF_{2\alpha}$ was found to be significantly increased in type 2 diabetic patients compared with age-matched healthy subjects. Furthermore, significant correlations were observed between blood glucose and urinary 8-iso-$PGF_{2\alpha}$, suggesting that the activation of oxidative stress may be, at least in part, related to the determinants of diabetic control. Such results are consistent with in vitro findings that demonstrated enhanced formation and release of 8-iso-$PGF_{2\alpha}$ by porcine vascular smooth muscle cells cultured under hyperglycemic conditions (27). More recently, we confirmed these results by comparing the urinary excretion rates of 8-iso-$PGF_{2\alpha}$ in type 2 diabetic patients with those observed in age-matched control subjects. The results showed that the mean urinary excretion rates of 8-iso-$PGF_{2\alpha}$ were significantly higher ($P < 0.01$) in patients with type 2 diabetes (means ± SD, 482 ± 206 pg/mg creatinine) than in nondiabetic healthy subjects (275 ± 85 pg/mg creatinine) (28).

Contribution of postprandial glucose to the activation of oxidative stress

The role of postprandial hyperglycemia in the generation of oxidative stress was particularly investigated by Ceriello (22), who demonstrated that the production of free radicals was increased during the postprandial period and that this increment was proportional to the magnitude of the postprandial glucose excursions. For instance, fasting nitrotyrosine, a metabolite derived from nitrosamine stress, was significantly increased in the diabetic patients. An additional increase was observed during postmeal periods. Reduction of the postmeal glucose excursions by using a premeal bolus of rapid insulin analog (aspart) resulted in parallel decrements in glycemic and nitrotyrosine responses (29). Such results have been confirmed by other studies (30).

Contribution of acute glucose fluctuations to the activation of oxidative stress

In a recent study (28), we demonstrated that the urinary excretion rate of 8-iso-PGF2α was highly and positively correlated with the glycemic variability assessed from the mean amplitude of glycemic excursions (MAGE) (31). For this purpose, the patients' glucose profiles were obtained over 48 h from continuous glucose monitoring system data. The calculation of the MAGE was made by measuring the arithmetic mean of the difference between consecutive peaks and nadirs, provided that the difference was greater than the SD around the mean glucose values. The relationship is indicated in Fig. 1 ($r = 0.86$, $P < 0.001$). A statistically significant correlation was also observed with the mean postprandial glucose incremental area under the curve, but the relationship was less significant ($P = 0.009$). It thus appears that the triggering effect of acute glycemic excursions on oxidative stress should be integrated into glycemic disorders that are much broader than acute postmeal spikes. As a consequence, the concept that postprandial hyperglycemic spikes are "dangerous waves" should be extended to both upward (postprandial) and downward (interprandial) acute fluctuations of glucose around a mean value. This observation may provide an explanation for some of the epidemiological observations of the Diabetes Control and Complications Trial. For instance, in the subgroups with a sustained A1C of 9% for the entire study duration, the risk of retinopathy was reduced by >50% in the intensive control group compared with the conventional group, even though these two subgroups of patients had the same A1C (7). The difference might have been due to a lower intraday glucose variability in the intensive control group. However, this hypothesis was not confirmed by a recent analysis of the datasets collected in the Diabetes Control and Complications Trial. In this retrospective study, Kilpatrick et al. (32) reported that the mean blood glucose (i.e., sustained chronic hyperglycemia) was predictive of microvascular complications in patients with type 1 diabetes, whereas within-day glucose variability was not. However, it should be noted that, in that study, the instability of blood glucose was calculated as the SD around the mean of a seven-point glycemic profile measured at each patient's quarterly visit. With such a methodology, the authors probably selected not major fluctuations but rather a composite of both major and minor swings with a majority of minor ones. Furthermore, Kilpatrick et al. (32) probably blunted the contributions of major glucose fluctuations because there are many reasons to think that the four preprandial (interprandial) and the three postprandial glu-

cose values included in the seven-point profile did not perfectly coincide with the glucose nadirs and peaks, respectively. The following example should be useful to explain the superiority of the MAGE index for assessing glucose variability compared with the SD of a seven-point glucose profile. Consider two patients with type 2 diabetes who have similar A1C and SD of glucose fluctuations around the mean. Assume that one subject has many minor glucose fluctuations and one or two major swings per day, whereas the other patient exhibits moderate glucose fluctuations over 24 h. Despite similar SD of glucose around the mean, these two patients should exhibit very different MAGE values, and thus Kilpatrick's use of SD as a definitive measure of glucose variability is questionable. Even though the MAGE determination requires continuous glucose monitoring, our opinion is that this index should be the gold standard for assessing glucose fluctuations in all prospective interventional trials designed to estimate glucose variability. Therefore, expanded use of continuous glucose sensors (33) would certainly be useful for conducting such trials.

To conclude this section, the pathophysiology of diabetes complications can be considered the result of two major deleterious metabolic alterations (excessive glycation and generation of oxidative stress) that are activated by three main glycemic disorders: hyperglycemia both at fasting and during postprandial periods and acute glucose fluctuations (Fig. 2). At present, there is no doubt that excessive levels of glucose at fasting and during postprandial periods activate the glycation process, which can be investigated as a whole by measuring the A1C levels. In addition to hyperglycemia at fasting, acute or sustained hyperglycemia over postprandial periods and more generally acute glucose fluctuations around the mean glucose value activate oxidative stress. The resulting effect is the risk of complications depicted by the diagonal arrow of a geometric cube for which three-dimensional coordinates on the three axes are fasting plasma glucose, postprandial glucose, and glucose fluctuations. According to this model, a global antidiabetic therapeutic strategy should be aimed at reducing the values of the three coordinates (i.e., the volume of the cube) and therefore the magnitude of the diagonal arrow that illustrates the risk for diabetes complications (Fig. 2).

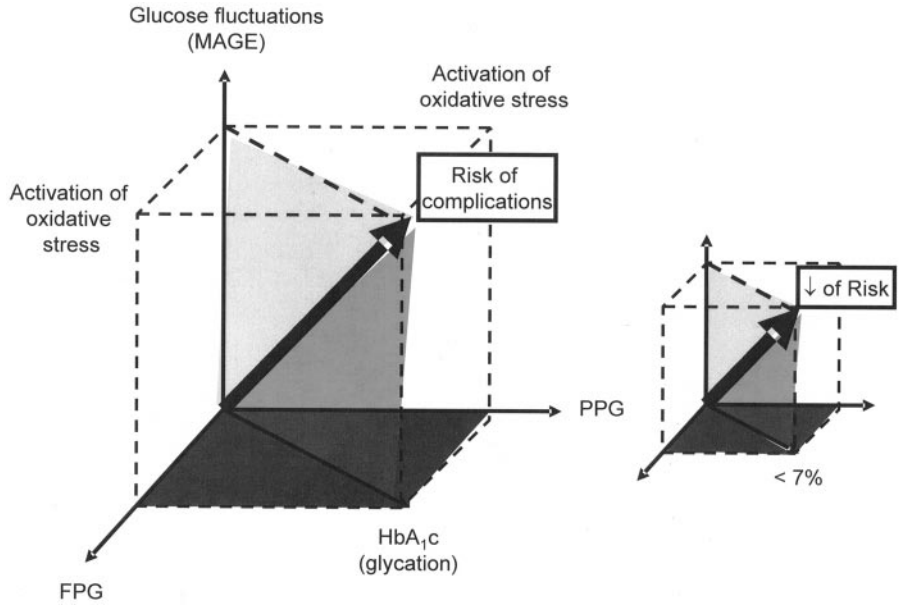

Figure 2—*Model suggested for illustrating the pathophysiological impacts of excessive glycation of proteins and activation of oxidative stress on the risk of diabetes complications (diagonal solid arrow). The contributions of the three components of dysglycemia, i.e., hyperglycemia at fasting (fasting plasma glucose [FPG]), hyperglycemia during postprandial periods (postprandial glucose [PPG]), and acute glucose fluctuations (MAGE), are indicated on the* x, y, *and* z *axes, respectively.*

GLYCEMIC VARIABILITY: CAN WE PREVENT IT? — This question is a subject of debate. The answer is probably yes, provided that the glucose variability is measured in interventional trials as well as in clinical practice. At present, most of the interventional trials using the "treat-to-target" concept have been conducted to achieve near-normal glucose value at fasting and A1C levels <7% (34). Postprandial and acute glucose fluctuations have rarely or never been taken into account. For this reason, it is difficult to estimate whether glucose variability can be reduced or not. To gain further insight into this problem, two examples can be considered. Both concern the treatment of patients with type 2 diabetes.

Can we prevent glucose variability by using long-acting insulin analogs?

In the LANMET (Lantus Metformin) study (35), the authors demonstrated that in insulin-naive type 2 diabetic patients who were not sufficiently controlled on oral antidiabetic agents, the implementation of a therapeutic strategy of combining either insulin glargine or NPH insulin with metformin resulted in similar improvements in A1C from baseline to end point at week 36. The mean A1C level dropped by ~2%, from 9% at baseline to 7% at end point in both groups. The eight-point glycemic profile was shifted downward, with the glycemic patterns exhibiting equal-sized changes from baseline to end point at different moments in the day, i.e., at fasting, interprandial, and postprandial time points. As a consequence and even though the glucose variability was not tested in this study, it seems from the glucose patterns that glucose variability remained unchanged and it can thus be concluded that the addition of a bedtime insulin dose failed to modify the acute glucose fluctuations from peaks to nadirs, whatever the type of insulin used, long or intermediate acting.

Can we prevent glucose variability by using glucagon-like peptide 1 agonists?

In suboptimally controlled patients with type 2 diabetes who were further treated with once-daily injection of insulin glargine, Heine et al. (36) observed that the changes in A1C and glycemic profiles were similar to those obtained by Yki-Järvinen et al. (35) in the LANMET study. In Heine's study (36), the glucose variability was not quantified, but the glucose fluctuations seemed to remain unchanged from baseline to end point in the glargine-treated group. In the second group (i.e., in patients who were treated with exenatide, a glucagon-like peptide 1 ago-

nist), the authors found that the improvement in A1C was the same as in the glargine-treated group. However, compared with the insulin glargine group, and everything else being equal in terms of glycemic disorders, the exenatide group exhibited less glucose variability at end point. As in the LANMET study, the magnitude of the glucose excursions was not quantified. However, by reading the crude differences between postprandial peaks and interprandial nadirs on the mean glucose patterns, it seems that the glucose variability was reduced by ~50% when the exenatide group was compared with the glargine group. Such data suggest that the implementation of treatments aimed at reducing postprandial excursions is certainly a valuable strategy for reducing the parameters that characterize the dysglycemia of diabetes (i.e., glycemic disorders both at fasting and during postprandial periods) and glycemic variability.

In conclusion, our opinion is that glucose variability should be one of the targets of treatment of the glycemic disorders encountered in patients with type 2 diabetes. Yet an important question remains to be answered: "Given that glucose variability is an important parameter, what exactly is the target? " The beginning of a response can be found in the relationship between the urinary excretion rate of 8-iso-$PGF_{2\alpha}$ and the MAGE (28) (Fig. 1). The analysis of the graph in Fig. 1 indicates that 275 pg/mg creatinine (i.e., the mean urinary excretion rate of isoprostanes in nondiabetic healthy subjects) corresponds to a MAGE value of 40 mg/dl. Therefore, 40 mg/dl can be proposed as the level of glucose variability that should be targeted, but it is obvious that further studies are necessary to define standards of glucose variability in diabetes.

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