Reduction of Oxidative Stress and Inflammation by Blunting Daily Acute Glucose Fluctuations in Patients With Type 2 Diabetes

Role of dipeptidyl peptidase-IV inhibition

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OBJECTIVE—Evaluate the effects of two dipeptidyl peptidase-IV (DPP-4) inhibitors, sitagliptin and vildagliptin, known to have different efficacy on mean amplitude of glycemic excursions (MAGE), on oxidative stress, and on systemic inflammatory markers in patients with type 2 diabetes.

RESEARCH DESIGN AND METHODS—A prospective, randomized, open-label PROBE design (parallel group with a blinded end point) study was performed in 90 patients with type 2 diabetes inadequately controlled by metformin. The study assigned 45 patients to receive sitagliptin (100 mg once daily; sitagliptin group) and 45 patients to receive vildagliptin (50 mg twice daily; vildagliptin group) for 12 weeks. MAGE, evaluated during 48 h of continuous subcutaneous glucose monitoring, allowed an assessment of daily glucose fluctuations at baseline and after 12 weeks in all patients. Assessment of oxidative stress (nitrotyrosine) and systemic levels of inflammatory markers interleukin (IL)-6 and IL-18 was performed at baseline and after 12 weeks in all patients.

RESULTS—HbA_{1c}, fasting and postprandial glucose, MAGE, and inflammatory and oxidative stress markers were similar between the groups at baseline. After 12 weeks, MAGE ($P < 0.01$) was lower in the vildagliptin group than in the sitagliptin group. After treatment, HbA_{1c} and postprandial glucose evidenced similar changes between the groups (P = NS). Vildagliptin treatment was associated with a stronger decrease in nitrotyrosine ($P < 0.01$), IL-6 ($P < 0.05$), and IL-18 ($P < 0.05$) than sitagliptin treatment. Nitrotyrosine and IL-6 changes significantly correlated with changes in MAGE but not in fasting glucose and HbA_{1c}.

CONCLUSIONS—MAGE reduction is associated with reduction of oxidative stress and markers of systemic inflammation in type 2 diabetic patients. These effects were greater in the vildagliptin group than in the sitagliptin group.

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Diabetes is characterized by the development of specific microvascular complications and a high incidence of accelerated atherosclerosis (1,2). Microvascular and macrovascular complications are mainly or partly (3–5) dependent on dysglycemia, which has two main components: chronic sustained hyperglycemia (integrated by HbA_{1c}) and acute glycemic fluctuations from peaks to nadirs (6). Both components lead to diabetes complications through two major mechanisms: activation of oxidative stress and increased activity of the innate immune system (7,8). Recent studies strongly suggest that daily periods of glucose fluctuations exhibited a more specific triggering effect on oxidative stress than chronic sustained hyperglycemia (9).

Oxidative stress has been highly and positively correlated with glycemic variability over a daily period as assessed from the mean amplitude of glycemic excursions (MAGE) (9). As consequence, the concept that postprandial hyperglycemic spikes are "dangerous waves" (10) should be extended to upward (postprandial) and downward (interprandial) periods as well as to nocturnal fluctuations of glucose around a mean value. All these values might be integrated in the MAGE. In such context, the failure of a therapeutic strategy targeting chronic sustained hyperglycemia to the normal levels in reducing cardiovascular events (11–13) might have been because the mere control of fasting glucose and HbA_{1c}, without control of glycemic excursions over a daily period, may be not sufficient to reduce oxidative stress and inflammation. Therefore, the pathophysiology of diabetes complications can be considered the result of three main glycemic disorders: fasting hyperglycemia, postprandial hyperglycemia, and acute glucose fluctuations over a daily period. Thus, a global antidiabetes therapeutic strategy should be aimed at reducing the values of those three main glycemic disorders.

It is not clear, however, whether pharmacologic interventions targeting glycemic excursions over a daily period provide specific benefits (reduction of oxidative stress and production of proinflammatory cytokines) relative to other pharmacologic therapies lowering HbA_{1c} comparably. The acute fluctuations of glucose around a mean value over a daily period have been proved independent of mean glycemia and related to defects in insulin secretion and suppression of glucagon secretion (14). More recently, we demonstrated that augmentation of glucagon-like peptide-1 (GLP-1) by inhibitors of the dipeptidyl peptidase-IV (DPP-4), such as vildagliptin, that enhance glucose-induced insulin secretion and decrease glucagon secretion over a daily period, reduces HbA_{1c} and glycemic fluctuations over a daily period (15). However, no prior studies have examined

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the effects of the blunted glycemic fluctuations with vildagliptin on atherosclerosis risk factors such as oxidative stress and proinflammatory cytokines.

According to the evidence that daily glucose fluctuations are more reduced in patients treated with vildagliptin (50 mg twice daily) than in patients treated with sitagliptin (100 mg once daily) (15), a study was conducted to evaluate the effects of vildagliptin as a therapeutic strategy useful for stabilizing glucose excursions over a daily period and lowering oxidative stress (as estimated from measurement of nitrotyrosine) and proinflammatory cytokines implicated in the atherosclerotic process, such as interleukin (IL)-6 and IL-18, in patients with type 2 diabetes poorly controlled along with metformin therapy.

RESEARCH DESIGN AND METHODS

We screened 111 type 2 diabetic patients regularly attending our clinic at the Second University of Naples. Among them, we selected 90 type 2 diabetic patients (43 men and 47 women) without adequate glycemic control (HbA_{1c} >7.5%) on metformin treatment at maximal dose (2,000 mg/day) for at least 8 weeks before enrollment. Criteria for exclusion encompassed insulin use or GLP-1 analog, concomitant chronic diseases, including kidney, liver, cardiovascular diseases, severe uncontrolled hypertension (blood pressure ≥200/100 mmHg), or recent acute illness, or a change in diet, treatment, or lifestyle within the 3 months before the study. All patients gave informed consent to participate in the trial, which was approved by our institution's ethics committee.

Randomized trial

We evaluated the effects of blunted daily glucose excursions on plasma nitrotyrosine, IL-6, tumor necrosis factor-α (TNF-α), and IL-18 levels in type 2 diabetic patients. The study was designed as a prospective, randomized, open-label PROBE (parallel group with a blinded end point) study of vildagliptin (50 mg twice daily; vildagliptin group) versus sitagliptin (100 mg once daily; sitagliptin group). The doses of both compounds are approved by the European Medicines Agency (EMA), and both compounds were planned to be used in a dose of 100 mg daily. Patients were informed about their therapy in both the vildagliptin and sitagliptin phases.

The study lasted 15 weeks, including a 3-week screening period and a 12-week active treatment period (Supplementary Fig. 1). After 3 weeks, eligible patients were equally randomized in a one-to-one ratio to one of two arms for 12 weeks. All patients received standard dietary counseling by a study investigator in accord with the dietary recommendations of the American Diabetes Association (16). The 90 patients who completed the 12-week study complied with the treatment program through the 6-week return for clinic visits and blood glucose monitoring. Patients were asked to record all hypoglycemic symptoms in their diaries and, if possible, to measure their blood glucose at the time of symptoms. The trial was conducted from May 2010 to June 2011.

Anthropometrics determination. Clinical evaluations included physical examination, vital signs, and review of adverse events. Fasting blood levels (at least 12 h from last meal) were assessed at every visit for glycemia and lipid profile, comprising total cholesterol, triglycerides, and HDL and LDL cholesterol. HbA_{1c} was measured at visits 1 and 3.

Continuous subcutaneous glucose monitoring. All patients underwent 48-h continuous subcutaneous glucose monitoring (CSGM) at visits 1 and 3. CSGM measurements were monitored for 3 consecutive days by using a CSGM system. The sensor was inserted on day 1 and removed on day 3 at midmorning. Glucose levels in a venous sample were calibrated on days 1 and 2 to determine fasting, postprandial, and interprandial glycemia. Standardized meal tests, comprising three mixed meals, was performed on days 1 and 2. After an overnight fast, patients received medications at 0700 h and had breakfast 30 min after treatment. Lunch and dinner were provided 5 and 10 h after the beginning of breakfast, respectively. Standardized breakfast contained 419 kcal (57% carbohydrate, 17% protein, and 26% fat), lunch contained 692 kcal (66% carbohydrate, 16% protein, and 18% fat), and dinner contained 507 kcal (41% carbohydrate, 26% protein, and 32% fat).

Glycemic evaluations. The glucose patterns obtained from each patient on study days 1 and 2 underwent the following readings and calculations: *1*) fasting glucose, mean of the fasting values (before breakfast); 2) postprandial glucose, mean of 2-h postmeal glucose; 3) insulin secretion (β-cell function), as the ratio between the area under the curve (AUC) of insulin and glucose over the meal (17); and *4*) MAGE, calculated according to Service et al. (18), was used for assessing glucose fluctuations during the daily periods. In particular, we used the glucose profiles obtained from the CGMS data on study days 1 and 2 (i.e., from continuous monitoring for 48 h). 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. Measurement of this parameter, which has been proved independent of mean glycemia, is of particular interest because the greater the MAGE, the higher the glycemic instability (19). After each meal, blood samples for measurement of plasma glucose, GLP-1, glucagon, and insulin were obtained every 30 min for 3 h. Values at 180 min after meals were considered as the interprandial period. The serum concentration of adiponectin levels was measured with enzyme-linked immunosorbent assay (R&D Systems). According to the homeostasis model assessment insulin resistance (HOMA-IR) was calculated as follows: Insulin resistance = fasting insulin (μU/mL) × fasting glucose (mmol/L)/22.5.

Oxidative stress. Nitrotyrosine plasma concentration was assayed by enzyme-linked immunosorbent assay. Nitrotyrosine was determined because this modified amino acid is a product of free-radical (O^{2-}) interaction with nitric oxide (NO). The interaction of O^{2-} with NO is very rapid and leads to inactivation of NO and production of the potent oxidant peroxynitrite. Detection of nitrotyrosine is strongly suggestive of increased generation of peroxynitrite (20). After an overnight fast, at breakfast time, and before the sensor insertion, venous blood samples were drawn for nitrotyrosine evaluation at visits 1 and 3.

Proinflammatory cytokines. Serum concentrations of IL-6 and IL-18 were determined in duplicate using a highly sensitive, quantitative sandwich enzyme assay (Quantikine HS PharmPak, R&D Systems). High-sensitivity TNF-α was assayed by immunonephelometry on a Behring Nephelometer 2 (Dade Behring, Marburg, Germany). After an overnight fast, at breakfast time, and before the sensor insertion, venous blood samples were drawn for the IL-6 and IL-18 evaluations at visits 1 and 3. Plasma C-reactive protein was determined using automated turbidimetry.

Statistical analysis

Data are presented as mean ± SD unless stated otherwise. Continuous variables were compared by the Student *t* test for independent variables. The incremental AUC for glucose, insulin, and glucagon was calculated by the trapezoidal method.

A cluster analysis allowed us to evaluate whether clustering of variables of inflammation was associated with fasting and prandial measures of glycemic control and β-cell function. For this purpose, we created a compound score, referred to as a clustering score. A *z* score quantifies the original score in terms of the number of SDs that score is from the mean of the distribution. It was calculated as the sum of the *z* scores of the main variable of inflammation (IL-6, IL-18, TNF-α). A *z* score indicates the position of an individual value of a variable in the total distribution of the variable in the population and is calculated as follows: (individual value – mean value)/SD.

Correlation analyses were performed using Pearson or Spearman correlation coefficients, as appropriate. Multivariate linear regression analyses were used to test the independent association of markers of glycemic control with nitrotyrosine and the inflammation score. A value of $P < 0.05$ was considered significant. All statistical analyses were performed by a single operator who was blinded to treatment group. All statistical analyses were performed with the use of SPSS software, version 12 software (SPSS Inc.).

RESULTS—Baseline characteristics of type 2 diabetic patients are given in Table 1 and Table 2. Anthropometric and clinical data were not different at baseline between the two study groups (Table 1). Basal glucometabolic data (HbA_{1c}, HOMA, fasting and postprandial glucose, and MAGE), values of inflammatory and oxidative stress markers, and adiponectin levels were similar in the two study groups (Table 2). Hormone profiles during standard meal and interprandial periods showed no differences during the prandial and interprandial period of active GLP-1 occurred in treatment with vildagliptin (twice daily) toward sitagliptin (100 mg once daily; Fig. 1).

In the whole population ($n = 90$), univariate analysis showed that nitrotyrosine levels correlated with MAGE ($r = 0.503$, $P < 0.001$) and with postprandial glucose values ($r = 0.299$, $P = 0.004$), whereas no correlations were found with HbA_{1c} ($r = -0.007$, P = NS) or fasting plasma glucose ($r = 0.057$, P = NS). In addition, MAGE correlated with fasting plasma IL-6 ($r = 0.409$, $P < 001$), IL-18 ($r = 0.50$, $P < 0.001$), and TNF-α ($r = 0.508$, $P < 0.001$). Postprandial glucose values correlated with levels of fasting plasma IL-6 ($r = 0.303$, $P < 0.001$), IL-18 ($r = 0.283$, $P < 0.001$), and TNF-α ($r = 0.257$, $P < 0.001$). In contrast, no correlations were observed between levels of HbA_{1c} and fasting plasma glucose and among fasting plasma IL-6, IL-18, and TNF-α (data not shown).

Significant correlation of MAGE ($r = 0.651$, $P < 001$; $n = 90$) and postprandial glucose ($r = 0.387$, $P < 001$; $n = 90$) with the inflammation score was also found. In contrast, the inflammation score did not correlate with HbA_{1c} and fasting plasma glucose or β-cell response (data not shown).

The independent associations of markers of glycemic control with nitrotyrosine and the inflammation score were tested in multivariate analyses. A model including age, BMI, HbA_{1c}, fasting plasma glucose, postprandial glycemia, β-cell response, and MAGE, as independent variables, explained 34% and 57% of nitrotyrosine and inflammation score variability, respectively. In such analyses, only MAGE ($\beta = 0.47$, $P < 0.001$; $\beta = 0.63$, $P < 0.001$) and postprandial glucose levels ($\beta = 0.34$, $P = 0.002$; $\beta = 0.41$, $P < 0.001$) were independently associated with both nitrotyrosine and the inflammation score, respectively.

Intervention study

After 3 months of therapy, 52.1% of the vildagliptin group and 47.9% of the sitagliptin group achieved a fasting glucose level <110 mg/dL (P = NS). No significant changes from baseline in lipid and blood pressure parameters occurred in either group (Table 2). Hypoglycemic events occurred in a similar number of patients in the vildagliptin ($n = 2$) and sitagliptin groups ($n = 3$).

Table 1—*Clinical characteristics of the study population* (n = 90)

	Sitagliptin group ($n = 45$)			Vildagliptin group ($n = 45$)		
	Baseline	After 12 weeks	*P*	Baseline	After 12 weeks	*P*
Age (years)	60 ± 8.5	—	NS	60 ± 8.8	—	NS
Sex (*n*)						NS
Male	20	—	NS	23	—	NS
Female	25	—	NS	22	—	NS
BMI (kg/m^2)	30 ± 5.7	29.1 ± 4.4	NS	29.7 ± 5.1	28.9 ± 3.9	NS
Waist-to-hip ratio	0.94 ± 0.09	0.93 ± 0.10	NS	0.95 ± 0.08	0.94 ± 0.09	NS
Diabetes duration (years)	8.6 ± 2.2	—	NS	8.9 ± 1.9	—	NS
Cholesterol-lowering drugs (%)	9	9	NS	7	7	NS
Antihypertensive drugs (%)	27	27	NS	28	28	NS
HbA_{1c} (%)	8.5 ± 1.1	7.3 ± 0.6	<0.001	8.2 ± 0.7	7.2 ± 0.5	<0.001
DBP (mmHg)	78 ± 8.9	77 ± 7.1	NS	79 ± 9.8	80 ± 10	NS
SBP (mmHg)	129 ± 15	128 ± 16	NS	132 ± 13	129 ± 18	NS
Cholesterol (mg/dL)						
Total	196 ± 43	194 ± 42	NS	199 ± 44	197 ± 44	NS
HDL	43 ± 12	45 ± 12	NS	46 ± 14	47 ± 13	NS
LDL	127 ± 40	126 ± 40	NS	121 ± 39	122 ± 39	NS
Triglycerides (mg/dL)	131 ± 57	127 ± 56	NS	148 ± 87	142 ± 86	NS

DBP, diastolic blood pressure; SBP, systolic blood pressure.

Table 2—*Glucose metabolism, oxidative stress, and inflammatory parameters before and 12 weeks after sitagliptin 100 mg once daily or vildagliptin 50 mg twice daily*

	Sitagliptin group (n = 45)			Vildagliptin group (n = 45)		
	Baseline	After 12 weeks	P	Baseline	After 12 weeks	P
Glucose (mg/dL)						
Fasting	146 ± 6.3	114 ± 6.8	<0.001	147 ± 6.8	116 ± 5.1	<0.001
2-h postprandial	216 ± 7.2	169 ± 5.41	<0.001	215 ± 7.3	169 ± 5	<0.001
HOMA-IR (index)	2.96 ± 0.61	2.65 ± 0.47	<0.005	3.09 ± 0.62	2.68 ± 0.52	<0.001
β-Cell responses (score)†	0.16 ± 0.007	0.25 ± 0.01	<0.001	0.17 ± 0.007	0.26 ± 0.01*	<0.001
Insulinogenic index	35 ± 2.5	38 ± 2.7	<0.001	35 ± 2.0	40 ± 3.9*	<0.001
Glucagon AUC ($mg \cdot h^{-1} \cdot dL^{-1}$)	11,737 ± 235	10,035 ± 641	<0.001	11,803 ± 353	9,220 ± 668*	<0.001
GLP-1 AUC ($pmol \cdot h^{-1} \cdot L^{-1}$)	1,487 ± 86	2,579 ± 63	<0.001	1,477 ± 98	3,280 ± 63*	<0.001
MAGE (mg/dL)	70.8 ± 21.6	64.75 ± 11.4	NS	73.9 ± 18.4	45.3 ± 16*	<0.001
Nitrotyrosine (μmol/L)	0.43 ± 0.06	0.36 ± 0.05	<0.01	0.42 ± 0.07	0.27 ± 0.03*	<0.001
IL-6 (pg/mL)	2.47 ± 0.41	1.95 ± 0.22	<0.001	2.47 ± 0.52	1.54 ± 0.16*	<0.01
IL-18 (pg/mL)	122 ± 9.2	110 ± 7.2	<0.001	124 ± 10.1	104 ± 5.5*	<0.01
TNF-α (pg/mL)	2.51 ± 0.11	2.38 ± 0.03	<0.001	2.53 ± 0.09	2.37 ± 0.052	<0.01
Inflammation (score)	−0.14 ± 2.14	1.22 ± 1.39	<0.001	0.14 ± 2.21	−1.22 ± 1.51*	<0.01
C-reactive protein (mg/dL)	1.08 ± 0.03	1.09 ± 0.18	NS	1.07 ± 0.09	1.04 ± 0.09	NS
Adiponectin (μg/mL)	9.2 ± 1.2	10.3 ± 1.4	NS	8.9 ± 1.2	9.8 ± 1.4	NS

*$P < 0.01$ compared with sitagliptin group. †β-Cell response = AUC insulin/AUC glucose.

Compared with baseline, sitagliptin and vildagliptin treatments both resulted in a significant decline in HbA_{1c}, HOMA-IR, and in fasting and postprandial glucose levels as well as in a nonsignificant increment of adiponectin levels, with no difference between the two groups. Indeed, vildagliptin, but not sitagliptin administration, was associated with a significant decline in MAGE (Table 2). Focusing on hormone profiles during standard meal and interprandial periods, a significant ($P < 0.05$) and greater increase during prandial and interprandial periods of active GLP-1 and β-cell response in vildagliptin (twice daily) toward sitagliptin (100 mg once daily) occurred (Fig. 1, Table 2). In addition, plasma glucagon levels were more suppressed during both the prandial and interprandial periods in subjects receiving vildagliptin compared with those receiving sitagliptin (Table 2).

Compared with baseline, treatment with sitagliptin or vildagliptin resulted in significantly lowered plasma IL-6, IL-18, TNF-α, and nitrotyrosine levels (Table 2). Indeed, vildagliptin versus sitagliptin resulted in greater reductions in IL-6, IL-18, and nitrotyrosine concentrations (Fig. 2) but not in plasma TNF-α and PCR levels (Table 2).

In the whole population (n = 90) and after 3 months therapy, changes in levels of plasma nitrotyrosine (r = 0.46, $P < 0.001$), IL-6 (r = 0.37, $P < 0.001$), IL-18 (r = 0.41, $P < 0.001$), and TNF-α (r = 0.447, $P < 0.001$) correlated with changes in MAGE values. Reduction in the IL-6 plasma level also correlated with decremental changes in postprandial glucose (r = 0.25, P = 0.01) and incremental changes in β-cell response (r = −0.26, P = 0.01) No correlations among changes in nitrotyrosine and cytokine plasma levels and changes in fasting glucose or HbA_{1c} were found (data not shown).

In the whole population (n = 90), inflammation score (r = 0.61, $P < 0.001$) significantly correlated with changes in MAGE value. Reduction in inflammation score also correlated with β-cell response (r = −0.25, $P < 0.001$). No correlation among changes in nitrotyrosine, inflammation score, and in changes in fasting glucose or HbA_{1c} was found (data not shown).

The independent association of changes in nitrotyrosine levels and inflammation score with changes in glucose parameters was evaluated by multivariate analysis. A model including age, BMI, ΔHbA_{1c}, Δfasting plasma glucose, Δpostprandial glycemia, Δβ-cell response, and ΔMAGE, as independent variables, explained 26% and 42% of nitrotyrosine and inflammation score variability, respectively. In such analyses, only the change in MAGE was independently associated with both nitrotyrosine (β = 0.39; $P < 0.001$) and inflammation score (β = 0.62; $P < 0.01$) changes.

CONCLUSIONS—Our study provides evidence that activation of oxidative stress and increased activity of the innate immune system can be reduced by the control of acute glucose swings over a daily period in type 2 diabetic patients. In particular, vildagliptin versus sitagliptin, despite similar plasma fasting hyperglycemia, HbA_{1c}, and postprandial glucose, was associated with a greater amelioration of MAGE and reduction of nitrotyrosine levels and proinflammatory cytokines. Although a nonglycemic effect (yet unknown) of vildagliptin on these atherosclerotic markers cannot be ruled out, these results suggest that excessive excursions of plasma glucose over a daily period are harmful for the vascular tree and that MAGE should be considered a treatment target of therapy.

We found that acute glucose fluctuations were strongly correlated with nitrotyrosine levels, whereas no relationship was observed when nitrotyrosine levels were plotted against the main markers of chronic sustained hyperglycemia (HbA_{1c} and fasting glucose concentrations). Nitrotyrosine is considered a good marker of peroxynitrite formation (20); thus, the strong correlation between nitrotyrosine levels and MAGE is strongly suggestive for increased generation of peroxynitrite, a potent oxidant. Because peroxynitrite is collectively formed from the interaction of O^{2-} with NO (21), the association between nitrotyrosine and MAGE may be a

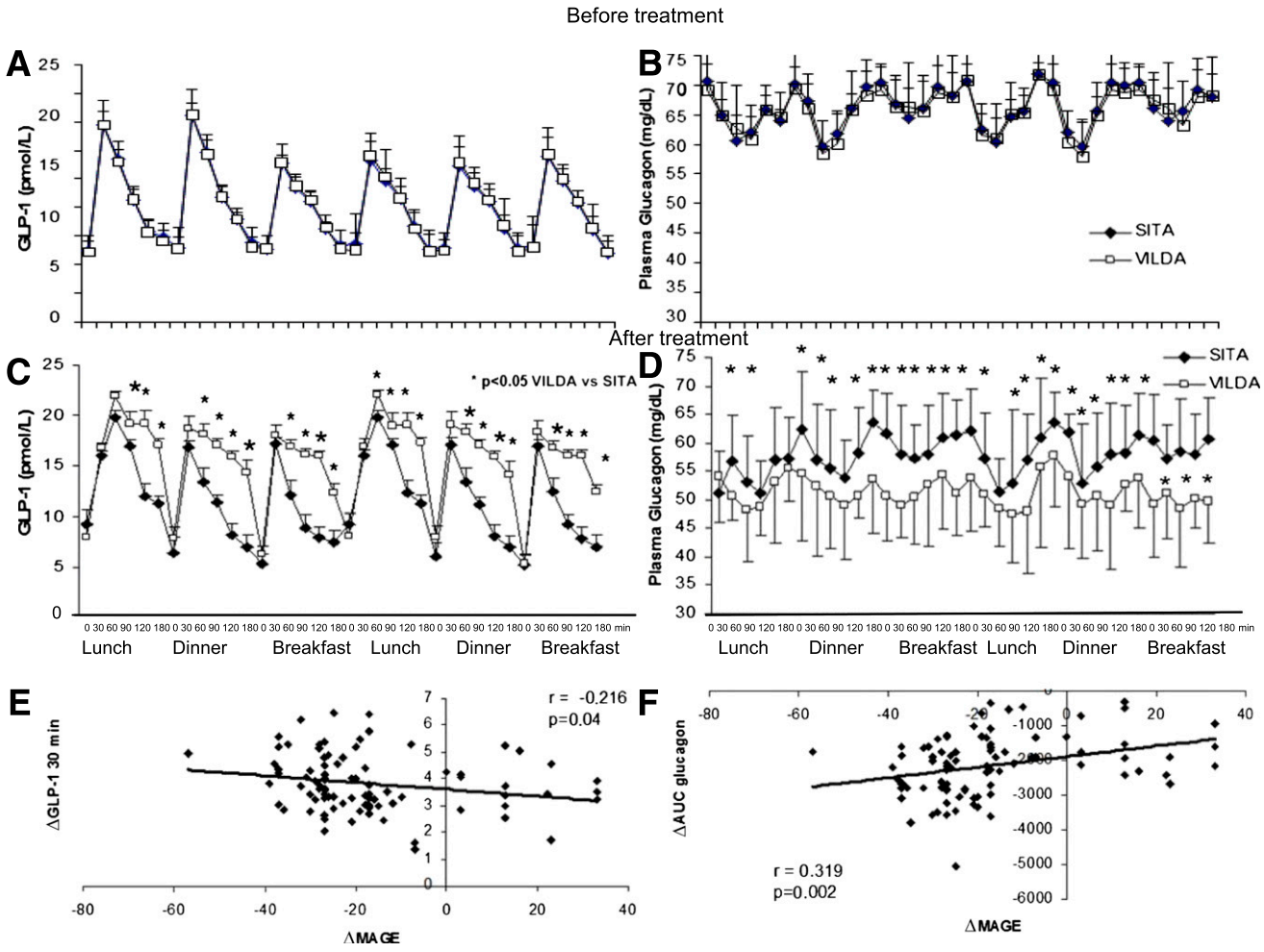

Figure 1—*Plasma GLP-1* (A) *and glucagon* (B) *levels are shown during standard meals at baseline in type 2 diabetic patients. Plasma GLP-1* (C) *and glucagon* (D) *levels are shown after 3 months of treatment with vildagliptin (50 mg, twice daily; VILDA) or sitagliptin (100 mg, once daily; SITA) in type 2 diabetic patients. The standardized breakfast contained 419 kcal (57% carbohydrate, 17% protein, and 26% fat), lunch contained 692 kcal (66% carbohydrate, 16% protein, and 18% fat), and dinner contained 507 kcal (41% carbohydrate, 26% protein, and 32% fat). Sample correlation analysis is shown between MAGE and GLP-1 at 30 min changes* (E) *and between MAGE and ΔAUC glucagon changes* (F). *Values are the mean ± SD.* *P < 0.05 *compared with the vildagliptin group.*

good indicator of the activation of oxidative stress as well as endothelial dysfunction in diabetic patients with acute glucose swings over a daily period, disorders that have been described as one of the main causes of vascular disease (21). A similar association was observed between MAGE and inflammatory markers, but no relationship was observed when IL-6 and IL-18 were plotted against the main markers of sustained chronic hyperglycemia (HbA_{1c} and fasting glucose concentrations). Such a profile of circulating cytokine concentration may be dangerous for cardiovascular health because elevated circulating concentrations of IL-6 predict future myocardial infarction among apparently healthy men (22), and elevated IL-18 concentrations predict future cardiovascular events and death in patients with documented coronary artery disease (23). In addition, we found that nitrotyrosine and circulating cytokines were also correlated with postprandial hyperglycemia values. However, our data evidenced that values of nitrotyrosine and cytokine were more dependent on MAGE than on postprandial glucose. Because glycemic fluctuations as estimated from MAGE indexes reflect both upward and downward glucose changes, whereas postprandial values are only markers of upward variations, there is some reason to think that MAGE indexes are wider integrators of glycemic variations than postprandial glucose. In addition, low glycemic levels in type 2 diabetes might stimulate oxidative stress and the innate immune system (24). Therefore, our data provide an important issue on the toxicity of daily glycemic excursions that can lead to endothelial damage as well as to microvascular and macrovascular complications.

Although several studies have found associations of oxidative stress with daily glucose swings (9,25), interventional studies have not evaluated the effects of the blunted MAGE on both oxidative stress and circulating cytokines. To our knowledge, this is the first demonstration that glucose fluctuations over a daily period affect the concentration of plasma cytokines and that the control of MAGE reduces the markers of systemic inflammation as well as oxidative stress in type 2 diabetic patients. However, a recent study suggests that a DPP-4 inhibitor exerts antiatherosclerotic effects and reduces inflammation via inhibition of monocyte activation/chemotaxis in mice with

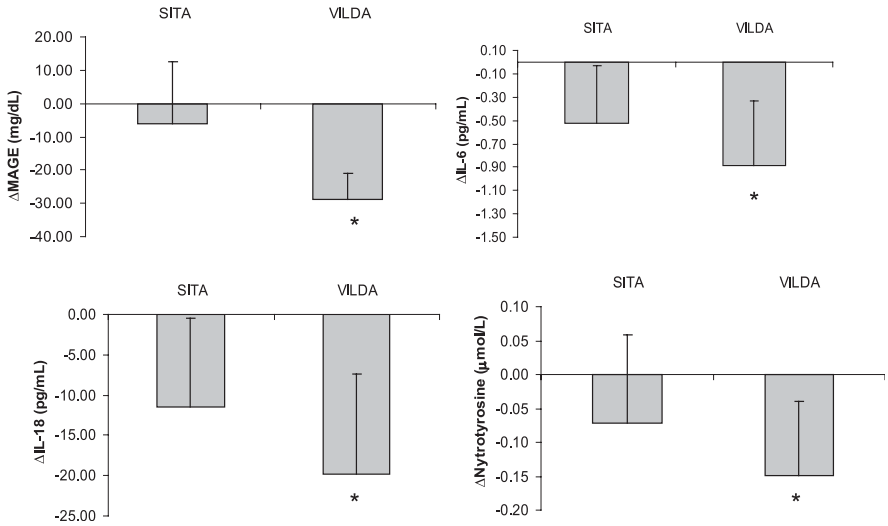

Figure 2—*Changes in MAGE and in plasma nitrotyrosine, IL-6, and IL-18 levels in vildagliptin (VILDA) and sitagliptin (SITA) group are shown after 3 months of therapy.* *$P < 0.05$.

atherosclerosis and insulin resistance (26). In our study, diabetic patients assigned to vildagliptin treatment obtained the greatest reduction in glycemic swings over a daily period. However, the efficacy of vildagliptin was comparable with sitagliptin on the control of main glucose parameters, such as HbA_{1c} and fasting and postprandial plasma glucose, over a 3-month study period; nevertheless, the effect to reduce glucose fluctuations over a day, as estimated from MAGE indexes that reflect upward and downward glucose changes, were more pronounced in the vildagliptin group than in the sitagliptin group. These effects could be due to a significantly a better daily GLP-1 inhibition profile, which could be responsible for the changes in MAGE within a shorter range.

The different kinetics of DPP-4 inhibition (vildagliptin acts as substrate inhibitor, whereas sitagliptin acts as competitive inhibitor) (27) may help to explain the different effects on incretin profile. On the basis of the crystal structures of chemically related pyrrolidine nitriles with DPP-4, vildagliptin is believed to form a reversible covalent imidate ester adduct, with the active site serine likely assisted by protonation by a neighboring residue, with morphologic change in the binding site, resulting in a longer and stable inhibition compared with sitagliptin, which binds to the same region of the protein as the pyrrolidine nitrile compounds, with the amide carbonyl of sitagliptin binding to Tyr547.62, without morphologic change in the binding site (27). Moreover, the differences in pharmacokinetic profiles may induce a different activity over a daily period: plasma DPP-4 activity is inhibited by almost 100% at 15–30 min, and >80% inhibition lasts for almost 14 h after a single 100-mg dose of sitagliptin (28); DPP-4 inhibition after vildagliptin (50 mg twice daily) remained >80% throughout the 24-h period (29).

Finally, it is possible that the further improvement observed with vildagliptin is due to a better bioavailability compared with sitagliptin: although the dosage of the two drugs is equivalent, vildagliptin is administered twice daily, whereas sitagliptin is administered once daily, as reported by EMA advice. Thus, the different DPP-4 inhibition profiles as well as the different pharmacokinetic profiles may be responsible for the different effects on glucose fluctuations over a daily period observed in diabetic patients treated with vildagliptin twice daily.

Moreover, patients who had the greatest reduction of MAGE had the largest reduction of both nitrotyrosine and IL-6 levels. Emerging data suggest that besides being a marker of cardiovascular risk, IL-6 may be a mediator of atherogenesis by quenching NO availability (30). Interestingly enough, glycemic excursions over a daily period also reduce NO bioavailability and increase peroxynitrite, pointing to the intriguing possibility that the combined effect of raised IL-6 concentrations and increased daily glycemic excursions levels in diabetic patients may act synergistically to diminish NO bioactivity. Moreover, increased inflammation and oxidative stress are observed in type 1 and type 2 diabetes, and interestingly, reduction of glycemic variability with pancreas transplantation almost completely eliminates oxidative stress (31,32). Finally, DPP-4 inhibition, with both vildagliptin and sitagliptin, had no effects on HOMA-IR and adiponectin levels, according to previous data (33).

Nevertheless, this study has some limitations. First, the randomized clinical trial used open-label administration of the study drug; however, the concealment of allocation and the use of an objective, blinded, end-point assessment strengthened the significance of results. Second, because of the limited follow-up, we could not evaluate clinical events.

In conclusion, in type 2 diabetic patients, amelioration of glycemic swings over a daily period provides superior efficacy for regression of atherosclerosis markers at 3 months compared with amelioration of both HbA_{1c} and fasting hyperglycemia. These results suggest that control of excessive daily glucose excursions may provide clinical benefit in type 2 diabetic patients, mostly in patients treated with vildagliptin, in which the control of glucose swings over a daily period were more evident than in the group treated with sitagliptin.

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M.R.R. researched data. M.B. researched data and contributed to discussion. R.M. researched data and wrote the manuscript. G.P. wrote, reviewed, and edited the manuscript. G.P. 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.

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