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Inhibition of Dipeptidyl Peptidase-4 Reduces Glycemia, Sustains Insulin Levels, and Reduces Glucagon Levels in Type 2 Diabetes

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The stimulation of insulin *vs.* inhibition of glucagon secretion in relation to the antidiabetic action of glucagon-like peptide-1 (GLP-1) is not established. Here, the influence of a 4-wk increase in circulating GLP-1 by inhibition of dipeptidyl peptidase-4 (DPP-4) on 24-h glucose and insulin and glucagon responses to breakfast was studied in subjects with dietary controlled diabetes [age: 65 ± 8 yr (SD), body mass index: 27.3 ± 3.3 kg/m^2, fasting plasma glucose: 9.0 ± 1.3 mmol/liter]. Compared with placebo (n = 19), a specific DPP-4 inhibitor [(1-[[(3-hydroxy-1-adamantyl) amino] acetyl]-2-cyano-(S)-pyrrolidine) (LAF237); 100 mg daily, n = 18] reduced fasting glucose by 0.70 mmol/liter ($P = 0.037$), 4-h prandial glucose excursion by 1.45 mmol/liter ($P < 0.001$), and mean 24-h glucose by 0.93 mmol/liter ($P < 0.001$). Baseline and postprandial active GLP-1 were increased by LAF237. The glucagon response to breakfast was reduced by LAF237 (glucagon levels at 60 min were 88 ± 8 pg/ml before treatment *vs.* 77 ± 5 pg/ml after; $P = 0.001$). In contrast, the overall insulin levels were not altered. The 4-wk reduction in glucagon correlated with the reduction in 2-h glucose ($r = 0.61$; $P = 0.008$). No such association was observed for insulin. Thus, improved metabolic control by DPP-4 inhibition in type 2 diabetes is seen in association with reduced glucagon levels and, despite the lower glycemia, unaltered insulin levels. (*J Clin Endocrinol Metab* 89: 2078–2084, 2004)

GLUCAGON-LIKE PEPTIDE-1 (GLP-1) stimulates insulin secretion, inhibits glucagon secretion, delays gastric emptying, induces satiety, and may increase islet cell mass (1–4). These effects contribute to the antidiabetogenic action of GLP-1 (5–8). The inhibition of glucagon secretion might be of particular importance because glucagon is a main determinant of glucose production from the liver (9), and type 2 diabetes is associated with hyperglucagonemia and defective suppression of glucagon secretion by glucose (10–14).

To develop the antidiabetogenic action of GLP-1 is difficult due to its rapid inactivation by the enzyme dipeptidyl peptidase-4 (DPP-4) (15, 16). Three approaches are possible: the continuous iv or sc infusion of GLP-1 (5, 6), the use of DPP-4-resistant GLP-1 analogs (3), or the use of compounds that inhibit the activity of DPP-4 (17). This latter approach has been successful in experimental studies in rodents (18–21) and recently, a 4-wk study in subjects with type 2 diabetes showed that a specific DPP-4 inhibitor improved glycemic control, verifying the potential of the DPP-4 concept in humans (22). It is not known, however, how DPP-4 inhibition affects insulin *vs.* glucagon levels along its improvement of metabolic control.

Therefore, in the present study, we have examined the influence of a 4-wk increase of active GLP-1 levels by means of DPP-4 inhibition on insulin and glucagon responses to a standardized meal in relation to the improvement of glucose tolerance in subjects with type 2 diabetes. As DPP-4 inhibitor, we used a potent, orally active and highly selective DPP-4 inhibitor, (1-[[(3-hydroxy-1-adamantyl) amino] acetyl]-2-cyano-(S)-pyrrolidine), called LAF237 (23).

Subjects and Methods

Study design

The study comprised a 4-wk run-in phase followed by a 4-wk double-blind treatment period during which patients were randomized to receive either placebo or LAF237 at 100 mg once daily, given 30 min before breakfast. LAF237 was manufactured under Good Manufacturing Practice in Novartis Pharmaceuticals (Basel, Switzerland) facilities. Four centers in Sweden participated (the University Hospitals in Lund, Malmö, Göteborg, and Umeå). The study was approved by the Ethics Committees at these institutions and by the Swedish Medical Products Agency (Uppsala, Sweden). Written informed consent was obtained from each participant. At wk 0 and 4, the subjects underwent a 24-h evaluation with standardized meals and frequent blood sampling. During these days, patients received a standard meal schedule comprising a total of 2000 kcal, consisting of 55% carbohydrate, 25% fat, and 20% protein (23% consumed at breakfast, 36% consumed at lunch, 36% consumed at dinner, and 5% consumed as a late evening snack). Breakfast consisted of 450 kcal with 50% as carbohydrate, 22% as fat, and 28% as protein. Participants were also provided with glucometers and instructed to monitor their blood glucose levels if they experienced symptoms of hypoglycemia.

Abbreviations: DPP, Dipeptidyl peptidase-4; GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide-1; HbA_{1c}, glycosylated hemoglobin; LAF237, (1-[[(3-hydroxy-1-adamantyl) amino] acetyl]-2-cyano-(S)-pyrrolidine); NS, not significant; PACAP, pituitary adenylate cyclase activating polypeptide.

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Subjects

Patients randomized to treatment were men or nonfertile women aged more than 30 yr with a history of type 2 diabetes for at least 12 wk. Nonfertile women were postmenopausal, posthysterectomy, or sterilized by tubal ligation. They had a mean fasting plasma glucose concentration in the range of 7.2–10.0 mmol/liter, a mean glycosylated hemoglobin (HbA_{1c}) level of 6.3–10% (reference value: <5.3%), fasting C-peptide levels of more than 0.3 nmol/liter, and a body mass index of 20–32 kg/m^2. No patient had evidence of type 1 diabetes, diabetes resulting from pancreatic injury, secondary forms of diabetes, a history of acute metabolic diabetic complications, or significant diabetic or other clinically relevant conditions (for example, asthma, myocardial infarction, or hyperlipidemia). Forty-six patients were screened but not randomized, mainly because diagnostic/severe criteria were not met (56.5%), and unacceptable laboratory values were recorded (39.1%). Of the patients randomized, three patients discontinued treatment due to withdrawal of consent (one patient), protocol violation (one patient), and administrative problem (one patient). Table 1 shows the clinical characteristics of the final 37 subjects who were studied. The characteristics did not differ significantly between the two groups. During the 4-wk treatment period, the patients adhered to their usual diet.

Analyses

Samples for analyses of DPP-4 activity were taken in tubes containing sodium heparin, and samples for analyses of GLP-1 and glucagon were taken in EDTA tubes with the addition of diprotin A (GLP-1) or aprotinine (glucagon). Samples for determination of glucose were taken in fluoride oxalate tubes, and samples for measurement of insulin were taken in serum separation tubes. DPP-4 activity was determined using an enzymatic method using the H-gly-pro-7-amino-4-methylcoumarin substrate. The method is based on the ability of DPP-4 to degrade H-gly-pro-7-aminomethylcoumarin to 7-amino-4-methylcoumarin, which is fluorescent. DPP-4 activity is expressed as milliunits of activity per milliliter plasma per minute of incubation with the substrate. Glucose was measured by the glucose oxidase method (Roche Molecular Biochemicals, Mannheim, Germany), insulin was measured by a RIA (Roche Molecular Biochemicals), glucagon was measured by RIA (Linco Research, Inc., St. Charles, MO), GLP-1 (active GLP-1 levels, *i.e.* $GLP\text{-}1_{7-36}$amide) was measured by ELISA (Linco), HbA_{1c} levels were determined by ion-exchange chromatography, and triglycerides and cholesterol levels were measured with enzymatic methods.

Safety and tolerability

Safety parameters included physical examination, vital signs, electrocardiographic evaluations, laboratory evaluations (hematology, chemistry, urinalysis), adverse events, and self-monitoring of blood glucose levels for suspected hypoglycemia. Symptomatic suspected hypoglycemic episodes were recorded even if not confirmed by a low blood glucose level. Asymtomatic low blood glucose levels (<2.5 mmol/liter) obtained on fingerstick were also recorded.

Statistics

Glycemia and insulinemia were evaluated as the mean 24-h glucose or insulin levels during the 24-h study periods, calculated as the area under the 24-h glucose or insulin curves divided by 24, fasting plasma glucose or insulin, the mean 4-h breakfast prandial glucose, calculated as the area under the 4-h glucose curve divided by 4, and the peak prandial glucose excursion after breakfast ingestion, defined as the difference between the maximum glucose values observed in the 4-h postmeal period minus the mean of the premeal measurements. All values are expressed as means ± SEM unless otherwise stated. Statistical comparisons were made between the LAF237 treatment group and the placebo group with the analysis of covariance modeling. Statistical comparison within the LAF237 treatment group between results before *vs.* after treatment was performed with paired Student's *t* test. Pearson's product moment correlation coefficients were obtained to estimate linear correlations between variables.

TABLE 1. Baseline clinical characteristics in LAF237 and placebo groups

Characteristic	Placebo (n = 19)	LAF237 (n = 18)
Gender (males/females)	10/9	13/5
Age (yr)	63.6 ± 7.3	66.9 ± 9.9
Body weight (kg)	81.8 ± 14.6	81.8 ± 9.8
BMI (kg/m^2)	27.1 ± 3.7	27.5 ± 3.0
Duration of diabetes (yr)	4.7 ± 4.5	4.6 ± 2.8
Fasting plasma glucose (mmol/liter)	9.2 ± 1.4	8.8 ± 1.5
Fasting plasma insulin (pmol/liter)	56 ± 29	53 ± 26
HbA_{1c} (%)	7.1 ± 0.7	7.2 ± 0.8

Means ± SD are shown.

Results

DPP-4 activity

Figure 1 shows the 24-h profile of DPP-4 activity before and after 4 wk of treatment with placebo or LAF237. At wk 4, LAF237 was taken 5 min after the 0 sample. In the sample taken at 15 min after ingestion of breakfast, *i.e.* 45 min after intake of LAF237, DPP-4 activity was reduced to 0.21 ± 0.04 mU/ml·min corresponding to 1.9 ± 0.1% of baseline activity ($P < 0.001$). Furthermore, in the sample taken 24 h after preceding intake of LAF237, DPP-4 activity was 7.3 ± 0.8 mU/ml·min *vs.* 10.9 ± 0.4 mU/ml·min in the placebo group, corresponding to 60 ± 5% of baseline activity ($P < 0.001$).

Mean 24-h glucose and insulin levels

Plasma glucose and insulin levels before and after 4 wk of treatment in the two groups are shown in Table 2 and Fig. 2. Before treatment, the glucose levels were very similar between the two groups. Treatment with LAF237 significantly reduced the mean 24-h glucose levels (by −1.45 ± 0.16 mmol/liter; placebo-controlled reduction was 0.93 mmol/liter; confidence interval: −1.38; −0.49 mmol/liter; $P < 0.001$). In contrast, mean 24-h insulin levels did not change

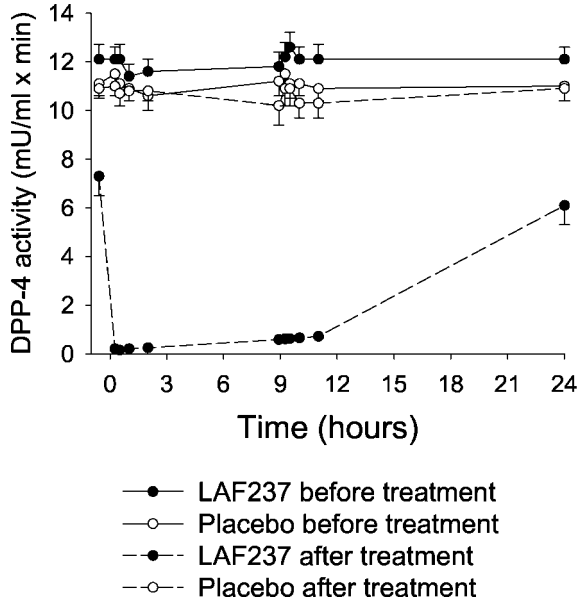

FIG. 1. Twenty-four-hour DPP-4 activity before and after 4 wk of treatment with placebo (n = 19) or LAF237 at 100 mg daily (n = 18) in subjects with type 2 diabetes. Means ± SEM are shown.

TABLE 2. Effect of 4-wk treatment with placebo (n = 19) or DPP-4 inhibition by LAF237 (100 mg daily; n = 18) in subjects with type 2 diabetes on fasting and prandial glucose and insulin levels

	Baseline at wk 0		Change from baseline at wk 4		Difference from placebo (CI)
	Placebo	LAF237	Placebo	LAF237	
Mean 24-h glucose (mmol/liter)	9.7 ± 0.5	9.2 ± 0.4	−0.5 ± 0.2	−1.5 ± 0.2	−0.9 (−1.4; −0.5) $P < 0.001$
Fasting glucose (mmol/liter)	9.2 ± 0.3	8.9 ± 0.3	−0.4 ± 0.2	−1.1 ± 0.2	−0.7 (−1.4; −0.1) $P = 0.037$
Mean 4-h breakfast prandial glucose (mmol/liter)	10.5 ± 0.5	10.0 ± 0.6	−0.4 ± 0.2	−1.9 ± 0.3	−1.5 (−2.2; −0.8) $P < 0.001$
Breakfast peak prandial glucose excursion (mmol/liter)	4.2 ± 0.37	4.5 ± 0.3	−0.3 ± 0.2	−1.3 ± 0.2	−1.0 (−1.6; −0.3) $P = 0.005$
Mean 24-h insulin (pmol/liter)	140 ± 15	128 ± 13	−13 ± 5	−20 ± 6	−7.5 (−23.6; 8.6) NS ($P = 0.356$)
Fasting insulin (pmol/liter)	56 ± 7	52 ± 6	0.4 ± 2.9	−7.2 ± 2.9	−7.6 (−16.0; 0.7) NS ($P = 0.073$)

Data are mean ± SEM. CI, 95% confidence interval of difference; *P*, probability level of random difference.

significantly in the group treated with LAF237 when compared with placebo.

GLP-1, glucose, insulin, and glucagon responses to breakfast

Figures 3 and 4 show the plasma levels of active GLP-1, glucose, insulin, and glucagon before and during the 120 min after ingestion of breakfast. Before the 4-wk treatment, no significant differences were seen between the groups for any of the variables (Fig. 3). After the 4-wk treatment, LAF237 increased active GLP-1 responses to breakfast and reduced glucose and glucagon responses to breakfast while having no effect on the insulin responses (Fig. 4). Baseline GLP-1 levels were significantly increased after treatment with LAF237 [3.7 ± 0.8 pmol/liter before treatment *vs.* 5.5 ± 1.0 pmol/liter after treatment ($P = 0.036$)]. Also, peak 30-min active GLP-1 levels after breakfast were increased by LAF237 [8.3 ± 0.8 pmol/liter before treatment *vs.* 16.5 ± 2.4 pmol/liter after treatment ($P < 0.001$)]. The fasting glucose, the mean 4-h prandial glucose, and the peak glucose level after breakfast were all reduced by LAF237 (Table 2). No significant effects were observed for fasting insulin, and the insulin response to meal ingestion was also not significantly affected by LAF237 (Table 2 and Fig. 2). The glucagon response to breakfast was reduced by LAF237. Thus, the peak 30-min glucagon was 102 ± 9 pg/ml in the LAF237 group before treatment; this was reduced to 90 ± 7 pg/ml after treatment ($P = 0.005$), and the corresponding numbers for the 60-min glucagon were 88 ± 8 pg/ml before treatment *vs.* 77 ± 5 pg/ml after treatment ($P = 0.001$). In contrast, no significant difference was observed in regard to glucagon response to breakfast for the placebo group, because the 30-min glucagon was 105 ± 3 pg/ml in the placebo group before treatment and 108 ± 8 pg/ml after treatment [not significant (NS)], and the corresponding numbers for the 60-min glucagon were 91 ± 6 pg/ml before treatment *vs.* 92 ± 6 pg/ml after treatment (NS).

Insulin vs. glucagon responses to breakfast vs. glucose tolerance

To examine the contribution of changes in insulin *vs.* glucagon for the improved glycemia after treatment with LAF237, the 60-min insulin and glucagon levels were correlated to the 2-h glucose values after breakfast. It was found that after treatment with placebo or LAF237, the 60-min glucagon correlated with the 2-h glucose levels (r = 0.51, $P = 0.001$; Fig. 5). Furthermore, the 4-wk reduction in the 60-min glucagon levels by LAF237 correlated with the 4-wk reduction in the 2-h glucose level (r = 0.61, $P = 0.008$). In contrast, corresponding insulin levels did not correlate to the 2-h glucose levels.

Body weight, HbA_{1c}, and lipids

Body weight was not altered significantly in any of the two groups during the 4-wk treatment period. The change in body weight was 0.12 ± 0.24 kg in the placebo group *vs.* 0.21 ± 0.23 kg in the LAF237 group (NS). HbA_{1c} was reduced by 0.15 ± 0.06% in the placebo group and by 0.53 ± 0.06% in the LAF237 group. The placebo-controlled change in HbA_{1c} was −0.38% (95% confidence interval: −0.54, −0.21; $P < 0.001$). No significant changes after LAF treatment were observed regarding total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, very low-density lipoprotein cholesterol, or triglycerides (data not shown).

Safety and tolerability

Treatment was well tolerated. In the LAF237 group, 12 patients reported adverse events, whereas in the placebo group, nine patients reported adverse events. The most common adverse events were nasopharyngitis (four patients in the LAF237 group *vs.* one patient in the placebo group), dizziness (two patients in the LAF237 group *vs.* three patients in the placebo group), headache (three patients in the LAF237 group *vs.* zero patients in the placebo group), and pruritis (two patients in each group). All adverse events were mild, and no treatment was discontinued. No patient experienced hypoglycemia.

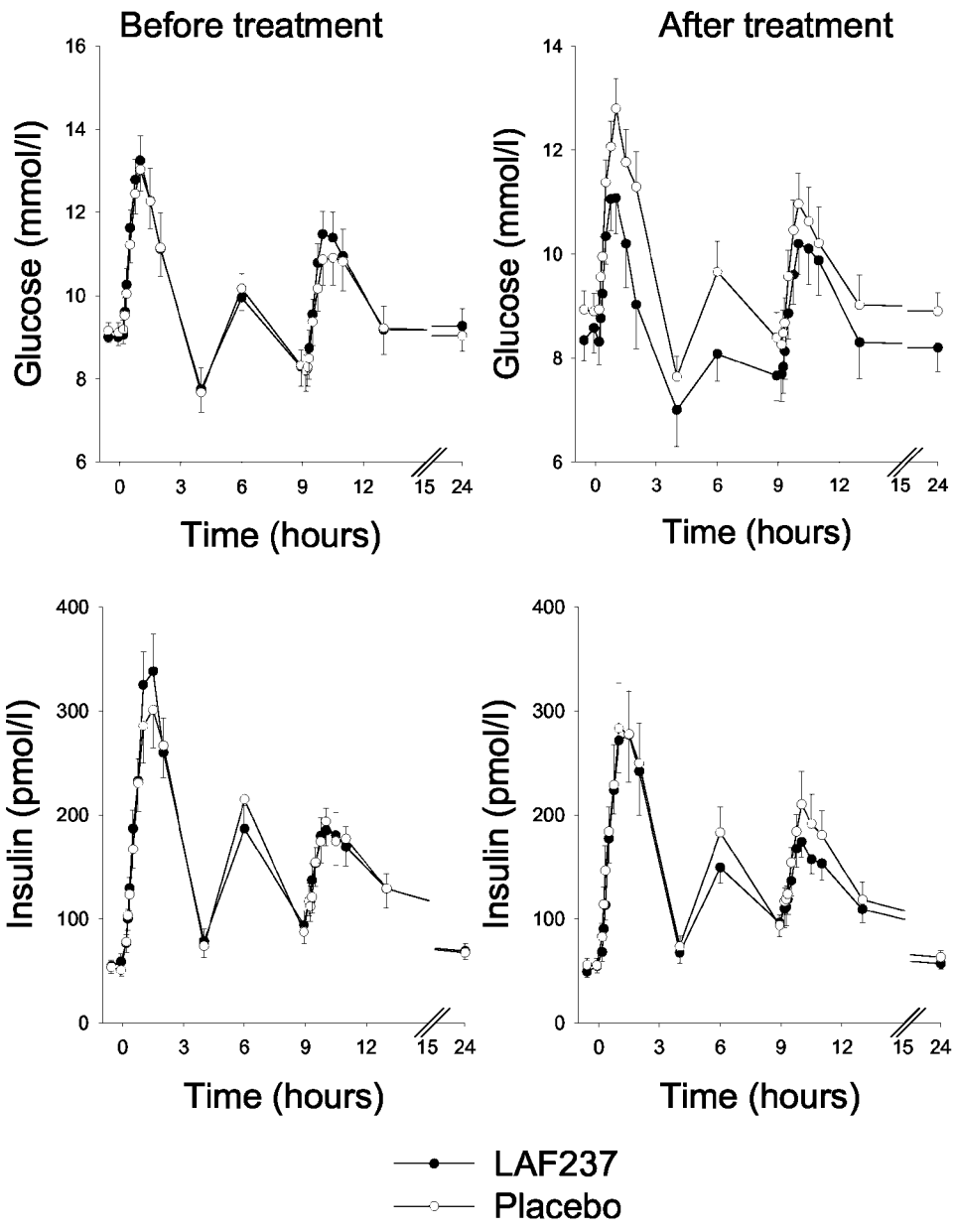

FIG. 2. Twenty-four-hour glucose and insulin levels before and after 4 wk of treatment with placebo (n = 19) or LAF237 at 100 mg daily (n = 18) in subjects with type 2 diabetes. Meals were ingested at time 0, 4 h 15 min, and 9 h, respectively. Means ± SEM are shown.

Discussion

This study examined the effect of 4-wk enhancement of GLP-1 activity due to DPP-4 inhibition on metabolic control and on insulin and glucagon responses to a standard meal in patients with mild type 2 diabetes. Patients were in a stable metabolic state after a 4-wk run-in period, and a parallel, placebo-treated control group allowed the effect of close clinic supervision to be identified and clearly separated from the drug effect. The effects we describe are, therefore, attributed to DPP-4 inhibition and not caused by an improved metabolic state secondary to, for example, better diet adherence.

The study shows that fasting glucose, mean 24-h glucose, and prandial glucose levels were significantly reduced by DPP-4 inhibition, whereas both prandial and mean 24-h insulin levels were not altered significantly. This would predict an improved insulin response to glucose although no statistically significant difference was found. A more pronounced effect, however, was evident on glucagon levels, with LAF237 significantly reducing the prandial glucagon response. This is probably of importance for the improved glucose tolerance after meal ingestion because the glucagon response to meal ingestion correlated with the glucose response, and the 4-wk reduction in the glucagon response to meal ingestion by LAF237 correlated with the 4-wk improvement in glycemia. Hence, subjects with more marked reduction in the glucagon response to meal ingestion had also the largest reduction in glucose. This may suggest that the reduction of the glucagon response to meal ingestion by DPP-4 inhibition is playing an important role in improving the glycemic response to meals after treatment with DPP-4 inhibition. This emphasizes the importance of absolute or relative hyperglucagonemia for the hyperglycemia in type 2 diabetes (10–14). The mechanism of the reduction in glucagon levels by DPP-4

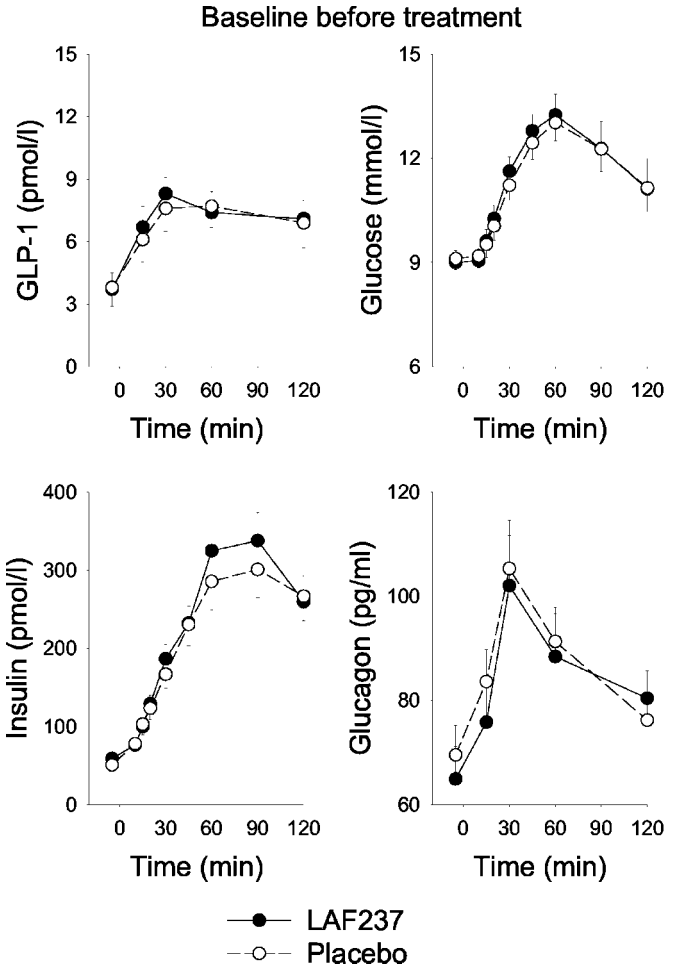

FIG. 3. Active GLP-1, glucose, insulin, and glucagon levels before and after intake of breakfast (performed at time 0) before treatment with placebo (n = 19) or LAF237 at 100 mg daily (n = 18) in subjects with type 2 diabetes. Means ± SEM are shown.

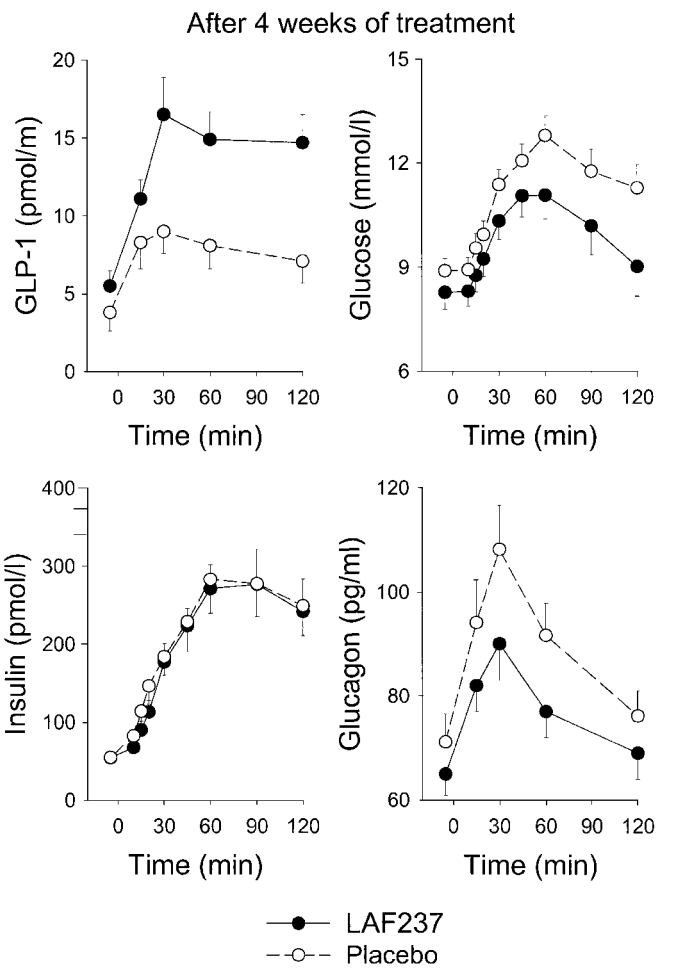

FIG. 4. Active GLP-1, glucose, insulin, and glucagon levels before and after intake of breakfast (performed at time 0) after 4 wk of treatment with placebo (n = 19) or LAF237 at 100 mg daily (n = 18) in subjects with type 2 diabetes. Means ± SEM are shown.

inhibition is not established from this study. One possibility is that DPP-4 inhibition delays gastric emptying, which will slow absorption of amino acids, thereby reducing the stimulation of glucagon secretion. The slightly slower increase in glucose levels after meal intake after DPP-4 treatment might suggest that delayed gastric emptying contributes to the effect. Another possibility is that DPP-4 inhibition results in inhibition of glucagon secretion at the level of the α-cells. Both of these effects, which need to be studied in more detail, may be mediated by GLP-1.

An important function of DPP-4 is to inactivate GLP-1 (15–17), and increased levels of active GLP-1 both at baseline and in response to meal were observed in our study. This would suggest that the effect of LAF237 to improve the glucose homeostasis is mediated by the increased active GLP-1 levels. However, contribution also by other bioactive peptides cannot be excluded because DPP-4 degrades also other peptides, which may be of importance in this context, such as glucose-dependent insulinotropic polypeptide (GIP) and pituitary adenylate cyclase activating polypeptide (PACAP) (24, 25). Both GIP and PACAP stimulate insulin secretion in humans (26, 27) and may thus be of importance. Such a hypothesis is also supported from experimental studies showing that DPP-4 inhibition reduces circulating glucose in mice with genetic deletion of the GLP-1 receptor (28), suggesting that preventing the inactivation of GLP-1 is not the sole mechanism for improved glycemia after DPP-4 inhibition. A difference exists between these peptides, however, because GLP-1 inhibits glucagon secretion (1–3), whereas both GIP (29) and PACAP (27) stimulate glucagon secretion in humans.

The combined effect of LAF237 to reduce fasting glucose yet maintaining fasting insulin unaffected might suggest that, in addition to the reduction of glucagon levels, an increased insulin sensitivity also might have developed during the 4-wk study period. This would be similar to the increase in insulin sensitivity seen after 6 wk of continuous sc infusion of GLP-1 (6). Such an effect is probably due to the overall improved metabolic control rather than to a direct effect of GLP-1, considering that GLP-1 has not been demonstrated to increase insulin sensitivity acutely in subjects with type 2 diabetes (30).

Of clinical importance is that the improved glycemia after 4 wk of continuous DPP-4 inhibition was associated with

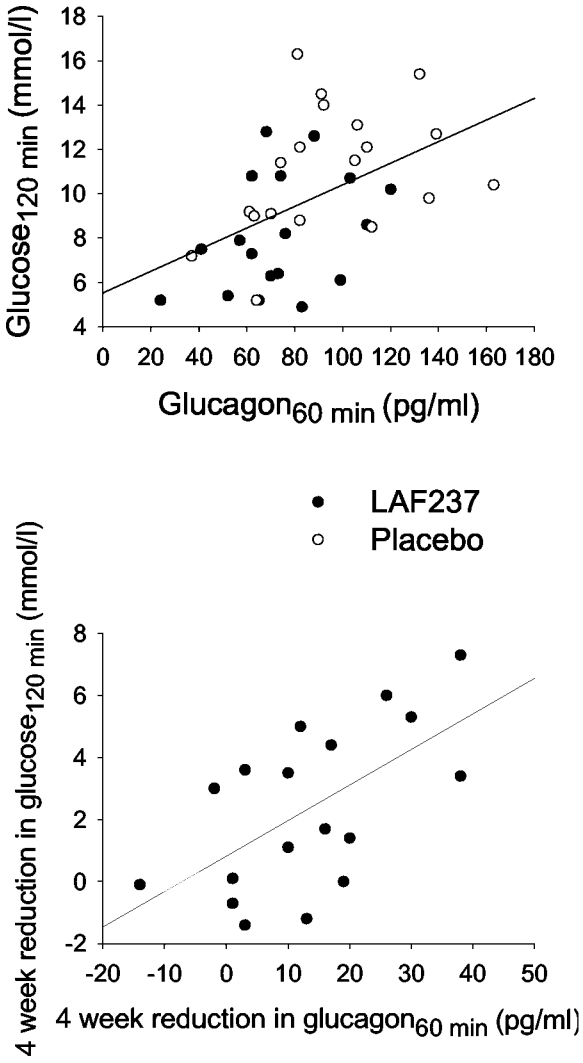

FIG. 5. *Upper panel*, Correlation between the 60-min glucagon levels and the 120-min glucose levels after ingestion of breakfast after 4 wk of treatment with placebo (n = 19) or LAF237 at 100 mg daily (n = 18) in subjects with type 2 diabetes. *Lower panel*, Correlation between the 4-wk reduction in 60-min glucagon and the 4-wk reduction in 120-min glucose after treatment with LAF237 at 100 mg daily (n = 18) in subjects with type 2 diabetes. Linear correlations are shown.

only few and mild adverse events, and, in particular, that body weight was not increased despite the improved glycemia. This is consistent with the aforementioned 6-wk study with GLP-1 infusion, where improved glycemia was observed without any increase in body weight (6).

In conclusion, this 4-wk study in subjects with mild type 2 diabetes shows that DPP-4 inhibition improves metabolic control, which is seen in association with reduced glucagon levels and, despite reduced glycemia, unaltered insulin levels. Whether DPP-4 inhibition is equally effective in more advanced stages of type 2 diabetes now remains to be studied.

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