Dipeptidyl-Peptidase-IV Inhibition Augments Postprandial Lipid Mobilization and Oxidation in Type 2 Diabetic Patients

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Context: Dipeptidyl-peptidase-IV (DPP-4) inhibition increases endogenous GLP-1 activity, resulting in improved glycemic control in patients with type 2 diabetes mellitus. The metabolic response may be explained in part by extrapancreatic mechanisms.

Objective: We tested the hypothesis that DPP-4 inhibition with vildagliptin elicits changes in adipose tissue and skeletal muscle metabolism.

Design and Setting: We conducted a randomized, double-blind, crossover study at an academic clinical research center.

Patients: Twenty patients with type 2 diabetes, body mass index between 28 and 40 kg/m^2, participated.

Intervention: Intervention included 7 d treatment with the selective DPP-4 inhibitor vildagliptin or placebo and a standardized test meal on d 7.

Main Outcome Measures: Venous DPP-4 activity, catecholamines, free fatty acids, glycerol, glucose, (pro)insulin, dialysate glucose, lactate, pyruvate, glycerol were measured.

Results: Fasting and postprandial venous insulin, glucose, glycerol, triglycerides, and free fatty acid concentrations were not different with vildagliptin and with placebo. Vildagliptin augmented the postprandial increase in plasma norepinephrine. Furthermore, vildagliptin increased dialysate glycerol and lactate concentrations in adipose tissue while suppressing dialysate lactate and pyruvate concentration in skeletal muscle. The respiratory quotient increased with meal ingestion but was consistently lower with vildagliptin.

Conclusions: Our study is the first to suggest that DPP-4 inhibition augments postprandial lipid mobilization and oxidation. The response may be explained by sympathetic activation rather than a direct effect on metabolic status. **(*J Clin Endocrinol Metab* 94: 846–852, 2009)**

Glucagon-like peptide-1 (GLP-1) and gastric inhibitory peptide (GIP) are released into the bloodstream from the intestinal tract wall in response to food intake. Both peptides are rapidly degraded to an inactive form by the enzyme dipeptidyl-peptidase-IV (DPP-4). Vildagliptin is a potent, selective, and orally active DPP-4 inhibitor that increases endogenous GLP-1

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Abbreviations: DPP-4, Dipeptidyl-peptidase-IV; GIP, gastric inhibitory peptide; GLP-1, glucagon-like peptide-1.

availability, resulting in improved glycemic control without increasing weight in patients with type 2 diabetes mellitus (1–5). The metabolic response to DPP-4 inhibition has been attributed to GLP-1-mediated increased glucose-mediated insulin and reduced glucagon secretion. However, the GLP-1 and GIP receptors are expressed not only in the pancreatic islets but also in the gastrointestinal tract, kidney, heart, lungs, central nervous system, and adipose tissue. For instance, vildagliptin decreases postprandial chylomicron apolipoprotein B-48 levels after 4 wk treatment, presumably due to either GLP-1 or GIP effects in the gut (6). Vildagliptin treatment for 6 wk is associated with reduced fasting fatty acid flux from adipose tissue (7). Because fasting insulin levels were not changed, this effect may also be an extrapancreatic effect of GLP-1 and/or GIP. In the same study, there was also an increase in insulin-mediated glucose disposal (presumably in muscle) that could not be ascribed to a direct pancreatic effect. Both the adipose and muscle responses could have been due an improvement in the overall metabolic state associated with the 6-wk treatment period (7). To determine whether or not there is a direct effect of vildagliptin on adipose tissue and skeletal muscle metabolism, patients in the present study were treated with vildagliptin for 7 d, and then tissue metabolism was monitored using the microdialysis technique.

Patients and Methods

Patients

Male or female patients aged 30–65 yr with type 2 diabetes and body mass index between 28 and 40 kg/m^2 were eligible to participate in the study. Patients had to have a glycosylated hemoglobin of 9% or lower at screening and documented fasting glucose of more than 125 mg/dl or more than 200 mg/dl at 2 h into the oral glucose tolerance test. Patients had to be able to complete a 3-wk washout of current antidiabetic medications. Patients taking medications that could alter gastric motility were not eligible. β-Adrenoreceptor blockers and lipid-lowering drugs had to be discontinued at least five half-lives before start of the study; other antihypertensive medications were permitted at a stable dose. Patients with a history of type 1 diabetes mellitus, secondary forms of diabetes, insulin therapy in the preceding 3 months, previous treatment with thiazolidinediones, significant concomitant disease or complications of diabetes, fasting triglycerides higher than 5.1 mmol/liter within the past 4 wk, or a history of gastrointestinal surgery, such as partial bowel resections, were excluded. The local ethics committee approved the study and all procedures were performed according to the revised Declaration of Helsinki and Good Clinical Practice requirements.

Study design

The study was a randomized, double-blind, crossover study comparing vildagliptin with placebo. Patients who were eligible for the study as determined at the screening visit were enrolled into a 21-d screening period. Patients who completed this period were then randomized to placebo or vildagliptin treatment for 7 d. They took 100 mg vildagliptin in the morning or matching placebo. On d six of the treatment period, patients were admitted to the inpatient facility where they ingested a light snack at 2200 h followed by an overnight fast. In the morning of d 7, patients were taken to the Clinical Research Center for the metabolic evaluation after ingestion of the last study drug dose. The first treatment period was followed by a 2-wk washout period. Then patients repeated the treatment phase such that patients who were first treated with placebo received vildagliptin and patients who were first treated with vildagliptin were given placebo.

Metabolic evaluation

Oxygen consumption and carbon dioxide production were measured by indirect calorimetry using a ventilated hood (DeltatracII; Datex Ohmeda, Duisburg, Germany) to assess resting energy expenditure and respiratory quotient. We calculated postprandial lipid and carbohydrate oxidation rates (8). A catheter was placed in a large antecubital vein for blood sampling. One microdialysis probe was inserted into abdominal sc adipose tissue, another one into skeletal muscle (quadriceps femoris, vastus lateralis) as described elsewhere (9, 10). We used CMA/60 microdialysis probes and CMA/102 microdialysis pumps (CMA Microdialysis AB, Solna, Sweden). After probe insertion, tissue perfusion with lactate-free Ringer solution (Serumwerke, Bernburg, Germany) was begun at a 2 μl/min flow rate. The perfusate was supplemented with 50 mmol/liter ethanol. After instrumentation, patients were allowed to recover for at least 60 min. Then they ingested the last dose of the study drug or placebo. Thirty minutes after drug intake, patients ate a standardized mixed meal with an energy content of 5 kcal/kg body weight (45% carbohydrates, 35% fat, 20% protein). Blood samples for the determination of glucose, free fatty acids, and insulin were obtained at baseline and 0.25, 0.5, 0.75, 1, 1.5, 2, 3, and 4 h after meal ingestion. Microdialysis samples were collected in 15-min intervals. We obtained additional blood samples after this period for determination of DPP-4 activity. We monitored blood pressure and heart rate using an oscillometric brachial blood pressure cuff (Dinamap; Criticon, Tampa, FL).

Analytical methods

DPP-4 was assayed by Novartis as described previously (11) using the H-Gly-Pro-7-amino-4-methylcoumarin substrate, which when enzymatically cleaved by DPP-4 produces fluorescent 7-amino-4-methyl coumarin. Production of fluorescent 7-amino-4-methyl coumarin is proportional to DPP-4 activity present in each sample. The concentrations of released 7-amino-4-methyl coumarin were converted into DPP-4 activity (milliunits per milliliter per minute). The lower level of quantitation for DPP-4 activity is 0.24 mU/ml · min. Plasma quality control samples were prepared at 7-amino-4-methylcoumarin concentrations of 1, 38.6, and 75.5 μmol/liter. The inter-day precision varied from 3.6–7.9%, and the accuracy varied from 96.4–107.2% of the nominal value. Active GLP-1 in plasma was determined using the GLP-1 (active) ELISA kit (Linco Research, Inc., St. Charles, MO). The lowest concentration of GLP-1 that could be detected was 2 pmol/liter. The inter-day coefficients of variation were 9.1% (6–12 pmol/liter) and 11% (27–56 pmol/liter). Catecholamines were collected in EGTA tubes (Kabevette; Kabe Labortechnik GmbH, Nümbrecht-Elsenroth, Germany) and processed immediately in a refrigerated centrifuge. The plasma was stored at −80 C until analysis. Plasma epinephrine and norepinephrine were assayed by HPLC with electrochemical (amperometric) detection (12). Microdialysis perfusate (inflow) and dialysate (outflow) ethanol concentrations were measured with a standard enzymatic assay. Dialysate glucose, lactate, pyruvate, and glycerol concentrations were measured with the CMA/600 analyzer. Changes in glycerol concentration were used to assess changes in lipolysis, and changes in glucose and lactate levels were used to assess changes in carbohydrate metabolism. *In situ* recovery for glycerol, glucose, and lactate in the dialysate was assessed by near-equilibrium dialysis (13). Recoveries of glucose, lactate, and pyruvate were

TABLE 1. Fasting measurements on the study day

	Vildagliptin	Placebo
Glucose (mmol/liter)	7.9 ± 0.53	8.1 ± 0.55
Free fatty acids (mmol/liter)	0.58 ± 0.04	0.58 ± 0.04
Glycerol (μmol/liter)	99 ± 6	97 ± 6
Triglycerides (mmol/liter)	2.1 ± 0.35	2.0 ± 0.27
Insulin (pmol/liter)	83 ± 9.7	83 ± 13
Proinsulin (pmol/liter)	8.0 ± 1.3	8.5 ± 1.3

None of the measurements was significantly different between placebo and vildagliptin (paired *t* test).

about 30% in adipose tissue and 50% in muscle. Recovery for glycerol was 30 and 80% in adipose tissue and muscle, respectively.

Statistical analysis

If not otherwise indicated, data are expressed as mean ± SEM. Differences between treatments were compared by paired *t* tests. Two-way ANOVA was used for multiple comparisons. In addition, we compared response curves after test drug administration with placebo treatment pairwise using global fitting. With this nonlinear regression method, one curve (placebo) for each subject is used as model (or baseline), allowing evaluations of discrepancy of the other curve (test drug). This method works in analogy to the paired-samples *t* test, using a pair of curves instead of a pair of values (14). *P* values <0.05 were considered statistically significant. All statistical tests were performed with GraphPad Prism version 4.0 (GraphPad Software Inc., San Diego, CA).

Results

We included 20 patients in our study (15 men, five women; age 55 ± 1 yr; body mass index 33 ± 0.4 kg/m^2). Basal laboratory data after patients had ingested placebo or vildagliptin for 6 d are given in Table 1. Fasting glucose, insulin, and free fatty acid measurements on the study day before the test meal and the last study medication dose were similar with placebo and with vildagliptin. DPP-4 activity and GLP-1 concentrations before and after ingestion of the last placebo or vildagliptin dose are illustrated in Fig. 1. DPP-4 activity was already reduced before ingestion of the last study drug dose. DPP-4 activity rapidly declined further after vildagliptin ingestion and remained unchanged on pla-

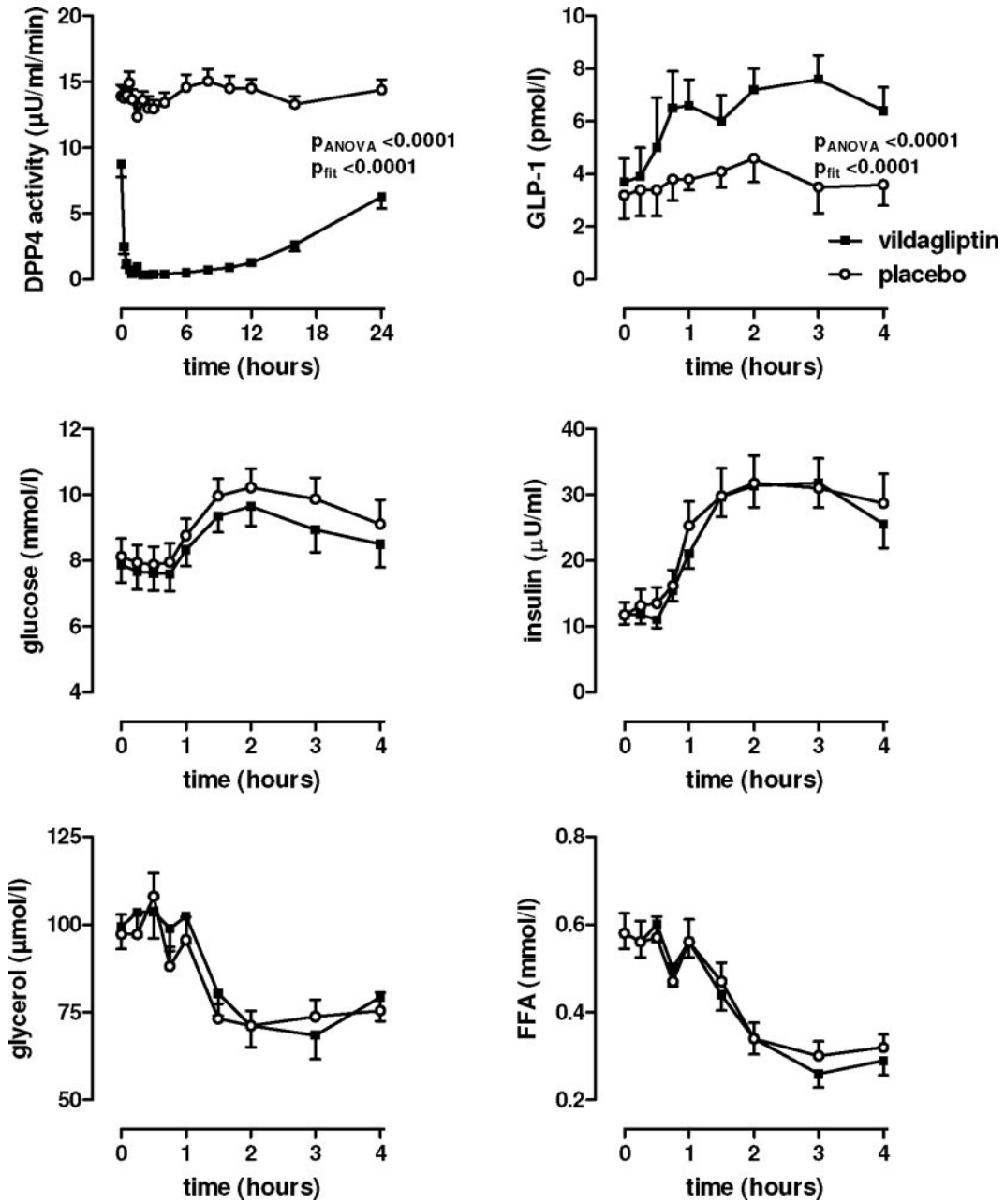

FIG. 1. Venous DPP-4 activity, GLP-1, glucose, insulin, glycerol, and free fatty acids (FFA) concentrations after patients ingested the last dose of the study drug in the morning (0 h) followed by a mixed meal. Please note that DPP-4 activity was measured over a 24-h period. P_{ANOVA} = *P* value by two-way-ANOVA; P_{fit} = *P* value for global fitting.

cebo. The response to vildagliptin was sustained during the day and abated after 24 h. DPP-4 inhibition was associated with a marked rise in circulating GLP-1 concentrations after ingestion of the test meal.

On placebo, insulin and glucose concentrations increased substantially after meal ingestion as shown in Fig. 1. Venous glycerol and free fatty acid concentrations were suppressed. Remarkably, all these responses were virtually identical after vildagliptin ingestion. Furthermore, before and after meal ingestion, venous triglyceride and proinsulin measurements were virtually identical with placebo and with vildagliptin. Vildagliptin augmented venous plasma norepinephrine, as shown in Fig. 2. Plasma epinephrine concentrations were almost identical with vildagliptin and with placebo and exhibited no increase upon food intake.

Figure 3 illustrates dialysate metabolite concentrations in adipose tissue and in skeletal muscle of patients treated with placebo and those treated with vildagliptin. Ethanol ratio in adipose tissue and in skeletal muscle at baseline and after meal ingestion was virtually identical with placebo and with vildagliptin. Dialysate glycerol concentrations in adipose tissue were increased with vildagliptin ($P_{ANOVA} < 0.0001$ between interventions) and similar with both interventions in skeletal muscle. Dialysate glucose concentrations in adipose tissue and in skeletal muscle before and after meal ingestion were virtually identical with vildagliptin and with placebo treatment. After meal ingestion, adipose dialysate lactate concentrations increased markedly on vildagliptin ($P_{ANOVA} < 0.0001$ between interventions). In the first 2 h after meal ingestion, muscle dialysate lactate increased similarly on placebo and on vildagliptin. Then, dialysate lactate continued to increase with placebo but remained stable on vildagliptin ($P_{ANOVA} < 0.0001$ between interventions). Similarly, muscle dialysate pyruvate concentrations increased more on placebo than on vildagliptin ($P_{ANOVA} < 0.0001$ between interventions).

Figure 4 illustrates changes in energy expenditure and respiratory quotient before and after meal ingestion. Energy expenditure tended to be increased with vildagliptin as suggested by a significant p-value for the global fitting analysis. However, the ANOVA *P* value was not significant. Respiratory quotient increased with meal ingestion but was consistently lower with vildagliptin. When we calculated postprandial lipid and carbohydrate oxidation rates, lipid oxidation rate was increased on vildagliptin.

Before ingestion of the last dose, blood pressure was 126 ± 3.2/76 ± 2.1 mm Hg with placebo and 124 ± 2.5/76 ± 1.5 mm Hg with vildagliptin. Four hours after ingestion of the test meal, blood pressure was 123 ± 2.7/75 ± 1.6 mm Hg with placebo and 123 ± 3.3/73 ± 2.0 mm Hg with vildagliptin. Moreover, heart rate before and after the meal was similar in both groups.

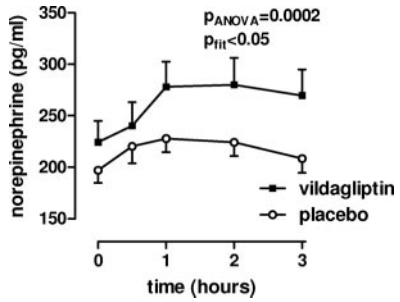

FIG. 2. Venous plasma norepinephrine concentrations after patients ingested the last dose of the study drug in the morning (0 h) followed by a mixed meal (n = 20). P_{ANOVA} = *P* value by two-way-ANOVA; P_{fit} = *P* value for global fitting.

Discussion

Our study suggests that augmentation of endogenous GLP-1 through DPP-4 inhibition facilitates postprandial lipid mobilization and oxidation. These effects are not likely to be due to an improved metabolic state because the patients were treated with vildagliptin for only 7 d. Although direct effects of GLP-1 or GIP are possible, it is more likely that they were secondary to the sympathetic activation that was associated with the enhanced lipid mobilization and oxidation.

DPP-4 inhibition with vildagliptin resulted in markedly increased postprandial venous GLP-1 concentrations. In contrast to GLP-1 mimetics, such as exenatide or liraglutide, DPP-4 inhibition results in a more physiological activation of the incretin system (15). GLP-1 has been previously shown to augment insulin release in a glucose-dependent fashion. Although vildagliptin results in improved insulin secretion relative to glucose stimulus, there is no increase in either fasting or postprandial insulin exposure during normal meals (1, 2, 16); increased insulin exposure is observed only after a 75-g glucose challenge (11). Thus, we were able to avoid the confounding effect of insulin in the current study, making data interpretation much easier.

Because lipolysis is under intense regulation by norepinephrine from adrenergic nerve endings in adipose tissue (17), we obtained serial plasma catecholamine measurements. Similar epinephrine concentrations with placebo and with DPP-4 inhibition exclude a major change in adrenal catecholamine release. Yet, we observed a moderate increase in venous plasma norepinephrine concentrations with DPP-4 inhibition compared with placebo. An increase in plasma norepinephrine could be explained by increased spillover of norepinephrine from sympathetic synapses or, less likely, reduction in norepinephrine clearance (18, 19). GLP-1 receptor activation in the brain has previously been shown to raise sympathetic activity in laboratory animals (20, 21). Furthermore, GLP-1 raises muscle sympathetic nerve activity in human subjects (22). In addition to GLP-1-mediated responses, DPP-4 interacts with the sympathetic nervous system through degradation of neuropeptide Y1–36 (23). Together, our and previous studies suggest an interaction between DPP-4 and the sympathetic nervous system, both in animals and in patients.

We assessed tissue metabolism using the microdialysis technique (24, 25). Dialysate metabolite concentrations are influenced by tissue perfusion, local metabolite production, and local metabolite utilization. To account for possible differences in tissue perfusion, we applied the ethanol dilution technique. The methodology can be applied in all non-ethanol-metabolizing organs including adipose tissue and skeletal muscle. Ethanol is added to the perfusion medium. Due to the concentration gradient, ethanol diffuses from the lumen of the microdialysis catheter into the interstitial space where it is removed via surrounding

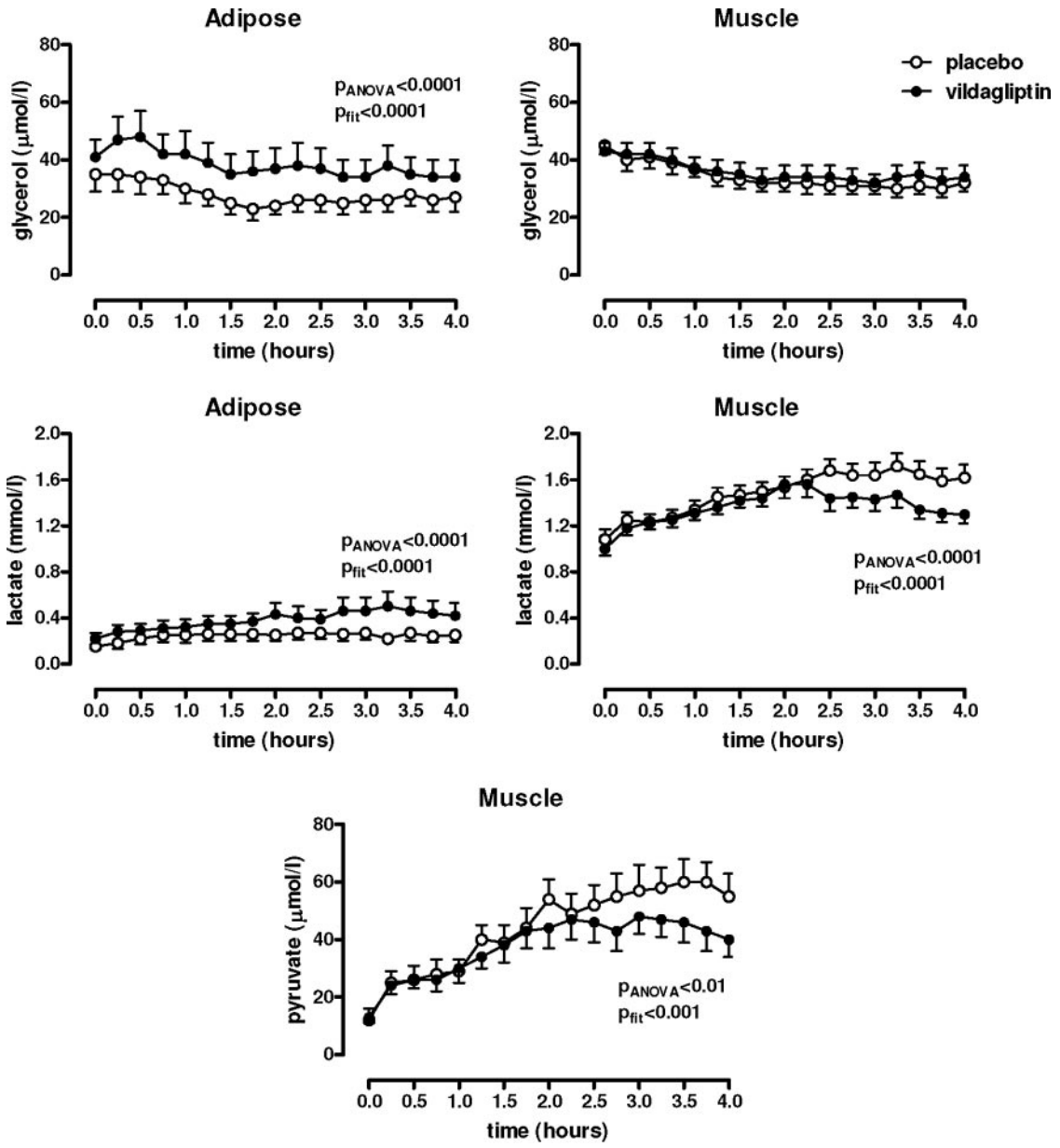

FIG. 3. Changes in dialysate glycerol and lactate in adipose tissue and in skeletal muscle and pyruvate in skeletal muscle after patients ingested the last dose of the study drug in the morning (0 h) followed by a mixed meal (n = 20). P_{ANOVA} = *P* value by two-way-ANOVA; P_{fit} = *P* value for global fitting.

capillaries. The higher the capillary blood flow the higher the removal of ethanol out of the tissue. Therefore, the ratio between ethanol concentration in the dialysate and in the perfusate is a useful semiquantitative measure of blood flow in the vicinity of the microdialysis catheter. A decrease in the ethanol ratio indicates an increase in tissue perfusion and an increase in that ratio the opposite. The ethanol dilution technique has been validated against direct blood flow measurements and xenon washout (26–28). Adipose tissue and skeletal muscle blood flow were similar with placebo and with vildagliptin both before and during ingestion of the test meal. The observation suggests that differences in dialysate metabolite concentrations are not explained by hemodynamic changes with DPP-4 inhibition.

The adipose tissue data are consistent with increased β-adrenergic stimulation leading to increased lipolysis and decreased triacylglycerol synthesis. Increased lipolysis is indicated by raised dialysate glycerol concentrations (25). Increased dialysate lactate levels in adipose tissue cannot be due to an increase in glucose transport because systemic glucose and insulin, dialysate glucose, and tissue blood flow before and during meal ingestion were similar with DPP-4 inhibition and with placebo. Instead, increased dialysate lactate indicates that a greater proportion of the glucose undergoes glycolysis. A smaller proportion is used for triacylglycerol synthesis. The net effect of both increasing lipolysis and decreasing triacylglycerol synthesis would be a raise of postprandial free fatty acid flux to other organs. Our observations do not support previous *in vitro* studies showing increased triacylglycerol synthesis with GLP-1 or GIP (29, 30). However, our measurements are indirect and do not completely rule out that locally mobilized fatty acids are reesterified in adipose tissue.

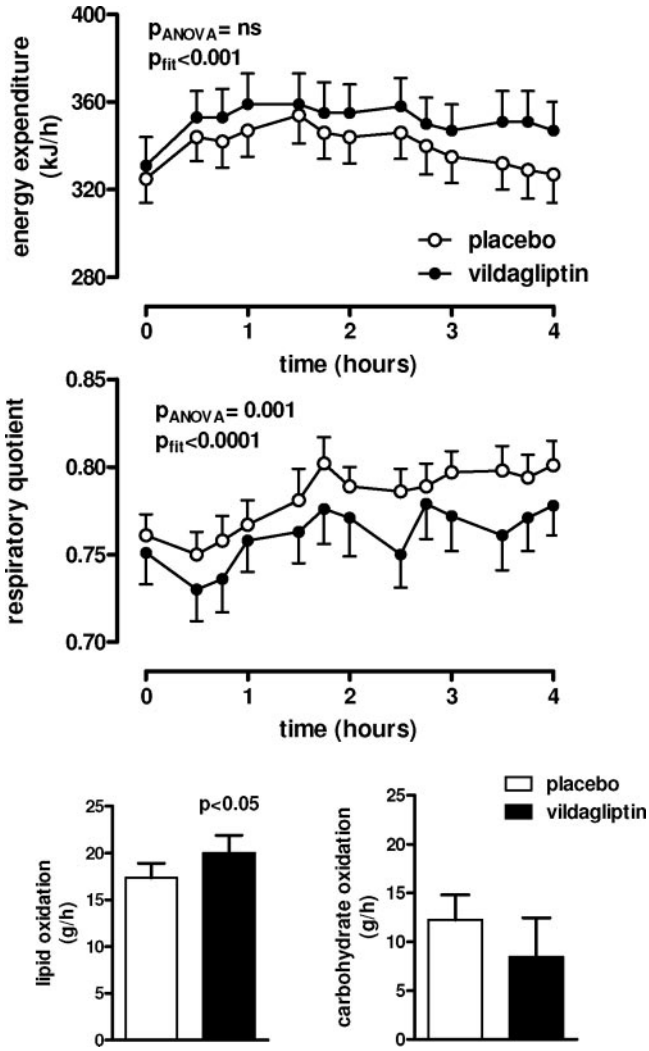

FIG. 4. Energy expenditure (*upper panel*), respiratory quotient (*middle panel*), and carbohydrate as well as lipid oxidation (*lower panel*) after patients ingested the last dose of the study drug in the morning (0 h) followed by a mixed meal (n = 20). P_{ANOVA} = *P* value by two-way-ANOVA; P_{fit} = *P* value for global fitting.

We observed a marked reduction in postprandial lactate and pyruvate production in skeletal muscle with DPP-4 inhibition. The observation cannot be explained by a decrease in glucose supply or in insulin release. Furthermore, the indirect calorimetry data showed a reduced carbohydrate oxidation and an increased lipid oxidation with DPP-4 inhibition. Sympathetically mediated fatty acid mobilization leads to increased free fatty acid levels, which in turn results in a shift from carbohydrate to fat metabolism in skeletal muscle (31). However, the fact that systemic free fatty acid concentrations were similar with placebo and with DPP-4 inhibition suggests that increased free fatty acid mobilization was matched by increased oxidation. In addition, an increase in sympathetic activity may have elicited a direct effect on muscular lipid oxidation independently of systemic free fatty acid concentrations (32). Possibly, reduced glucose oxidation and lactate production in skeletal muscle is compensated by more effective inhibition of hepatic glucose production by DPP-4 inhibition with vildagliptin (33).

Previously, studies suggested that more chronic DPP-4 inhibition decreases fasting fatty acid flux (7). The observation suggested that DPP-4 inhibition with vildagliptin would shift the balance toward increased lipid storage in fat cells and decreased lipid storage in muscle and liver. A similar mechanism has been demonstrated for the thiazolidinediones and appears to contribute to the antidiabetic effect of this drug class. Yet, our study suggests that DPP-4 inhibition may improve lipid oxidation rather than lipid storage in adipose tissue. An increase in lipid oxidation may be beneficial because reduced mitochondrial uptake and oxidation of free fatty acids has been linked with insulin resistance and type 2 diabetes mellitus (34, 35). Intramuscular accumulation of lipid intermediates, such as diacylglycerol and ceramides, may interfere with insulin signaling through increased serine phosphorylation of insulin receptor substrate-1 (IRS-1) (36). We speculate that reversal of incomplete fatty acid oxidation may contribute to increased insulin-mediated glucose disposal with DPP-4 inhibition (7).

Unlike GLP-1 mimetics, there is no satiety effect of DPP-4 inhibition with vildagliptin (37). The increased postprandial lipid oxidation reported here could increase postprandial thermogenesis and thus serve to explain the finding that vildagliptin does not lead to weight gain, which is invariably the case with insulin, sulfonylurea, and thiazolidinedione therapy (38).

We conclude that DPP-4 inhibition affects postprandial carbohydrate and lipid metabolism in a tissue-specific fashion. An increase in postprandial adipose tissue lipolysis is associated with augmented postprandial systemic lipid oxidation rate. We speculate that the metabolic response to DPP-4 inhibition is mediated through GLP-1 receptor-mediated sympathetic nervous system activation. This effect is unlikely to occur directly in the brain, because vildagliptin does not cross the blood-brain barrier. We are fortuitously able to exclude an insulin-mediated mechanism, because insulin responses did not differ between vildagliptin- and placebo-treated patients. Our findings suggest a novel DPP-4 inhibitor-mediated mechanism of action. Furthermore, DPP-4 inhibition augmented the renovascular response to angiotensin II in susceptible animals (23). We were reassured to observe no changes in blood pressure or heart rate with DPP-4 inhibition. In contrast, GLP-1 receptor activation within the pharmacological range substantially raised blood pressure in animals (20).

Acknowledgments

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References

1. **Brown J** 1962 Effects of 2-deoxyglucose on carbohydrate metabolism: review of the literature and studies in the rat. Metabolism 11:1098–1111
2. **Ahren B, Gomis R, Standl E, Mills D, Schweizer A** 2004 Twelve- and 52-week

efficacy of the dipeptidyl peptidase IV inhibitor LAF237 in metformin-treated patients with type 2 diabetes. Diabetes Care 27:2874–2880
3. **Dejager S, Razac S, Foley JE, Schweizer A** 2007 Vildagliptin in drug-naive patients with type 2 diabetes: a 24-week, double-blind, randomized, placebo-controlled, multiple-dose study. Horm Metab Res 39:218–223
4. **Garber AJ, Schweizer A, Baron MA, Rochotte E, Dejager S** 2007 Vildagliptin in combination with pioglitazone improves glycaemic control in patients with type 2 diabetes failing thiazolidinedione monotherapy: a randomized, placebo-controlled study. Diabetes Obes Metab 9:166–174
5. **Pi-Sunyer FX, Schweizer A, Mills D, Dejager S** 2007 Efficacy and tolerability of vildagliptin monotherapy in drug-naive patients with type 2 diabetes. Diabetes Res Clin Pract 76:132–138
6. **Matikainen N, Manttari S, Schweizer A, Ulvestad A, Mills D, Dunning BE, Foley JE, Taskinen MR** 2006 Vildagliptin therapy reduces postprandial intestinal triglyceride-rich lipoprotein particles in patients with type 2 diabetes. Diabetologia 49:2049–2057
7. **Azuma K, Radikova Z, Mancino J, Toledo FG, Thomas E, Kangani C, Dalla MC, Cobelli C, Holst JJ, Deacon CF, He Y, Ligueros-Saylan M, Serra D, Foley JE, Kelley DE** 2008 Measurements of islet function and glucose metabolism with the dipeptidyl peptidase 4 inhibitor vildagliptin in patients with type 2 diabetes. J Clin Endocrinol Metab 93:459–464
8. **Ferrannini E** 1988 The theoretical bases of indirect calorimetry: a review. Metabolism 37:287–301
9. **Jordan J, Tank J, Stoffels M, Franke G, Christensen NJ, Luft FC, Boschmann M** 2001 Interaction between β-adrenergic receptor stimulation and nitric oxide release on tissue perfusion and metabolism. J Clin Endocrinol Metab 86: 2803–2810
10. **Boschmann M, Engeli S, Adams F, Gorzelniak K, Franke G, Klaua S, Kreuzberg U, Luedtke S, Kettritz R, Sharma AM, Luft FC, Jordan J** 2005 Adipose tissue metabolism and CD11b expression on monocytes in obese hypertensives. Hypertension 46:130–136
11. **He YL, Wang Y, Bullock JM, Deacon CF, Holst JJ, Dunning BE, Ligueros-Saylan M, Foley JE** 2007 Pharmacodynamics of vildagliptin in patients with type 2 diabetes during OGTT. J Clin Pharmacol 47:633–641
12. **Willemsen JJ, Ross HA, Jacobs MC, Lenders JW, Thien T, Swinkels LM, Benraad TJ** 1995 Highly sensitive and specific HPLC with fluorometric detection for determination of plasma epinephrine and norepinephrine applied to kinetic studies in humans. Clin Chem 41:1455–1460
13. **Stahle L, Segersvard S, Ungerstedt U** 1991 A comparison between three methods for estimation of extracellular concentrations of exogenous and endogenous compounds by microdialysis. J Pharmacol Methods 25:41–52
14. **Motulsky H** 1995 Using nonlinear regression to fit curves. In: Intuitive biostatistics. New York: Oxford University Press; 277–283
15. **Hermansen K, Mortensen LS** 2007 Bodyweight changes associated with antihyperglycaemic agents in type 2 diabetes mellitus. Drug Saf 30:1127–1142
16. **Pratley RE, Jauffret-Kamel S, Galbreath E, Holmes D** 2006 Twelve-week monotherapy with the DPP-4 inhibitor vildagliptin improves glycemic control in subjects with type 2 diabetes. Horm Metab Res 38:423–428
17. **Bartness TJ, Song CK** 2007 Thematic review series: adipocyte biology. Sympathetic and sensory innervation of white adipose tissue. J Lipid Res 48:1655–1672
18. **Esler MD, Jennings G, Lambert G, Meredith I, Horne M, Eisenhofer G** 1990 Overflow of catecholamine neurotransmitters to the circulation: source, fate, and functions. Physiol Rev 70:963–985
19. **Meredith IT, Eisenhofer G, Lambert GW, Jennings GL, Thompson J, Esler MD** 1992 Plasma norepinephrine responses to head-up tilt are misleading in autonomic failure. Hypertension 19:628–633
20. **Yamamoto H, Lee CE, Marcus JN, Williams TD, Overton JM, Lopez ME, Hollenberg AN, Baggio L, Saper CB, Drucker DJ, Elmquist JK** 2002 Glucagon-like peptide-1 receptor stimulation increases blood pressure and heart rate and activates autonomic regulatory neurons. J Clin Invest 110:43–52
21. **Osaka T, Endo M, Yamakawa M, Inoue S** 2005 Energy expenditure by intravenous administration of glucagon-like peptide-1 mediated by the lower brainstem and sympathoadrenal system. Peptides 26:1623–1631
22. **Bharucha AE, Charkoudian N, Andrews CN, Camilleri M, Sletten D, Zinsmeister AR, Low PA** 2008 Effects of glucagon-like peptide-1, yohimbine, and nitrergic modulation on sympathetic and parasympathetic activity in humans. Am J Physiol Regul Integr Comp Physiol 295:R874–R880
23. **Jackson EK, Mi Z** 2008 Sitagliptin augments sympathetic enhancement of the renovascular effects of angiotensin II in genetic hypertension. Hypertension 51:1637–1642
24. **Ungerstedt U** 1997 Microdialysis in normal and injured brain. In: Kinney JM, Tucker HN, eds. Physiology, stress, and malnutrition. New York: Lippincott-Raven; 361–374
25. **Arner P, Bulow J** 1993 Assessment of adipose tissue metabolism in man: comparison of Fick and microdialysis techniques. Clin Sci 85:247–256
26. **Fellander G, Linde B, Bolinder J** 1996 Evaluation of the microdialysis ethanol technique for monitoring of subcutaneous adipose tissue blood flow in humans. Int J Obes Relat Metab Disord 20:220–226
27. **Hickner RC, Ekelund U, Mellander S, Ungerstedt U, Henriksson J** 1995 Muscle blood flow in cats: comparison of microdialysis ethanol technique with direct measurement. J Appl Physiol 79:638–647
28. **Hickner RC, Bone D, Ungerstedt U, Jorfeldt L, Henriksson J** 1994 Muscle blood flow during intermittent exercise: comparison of the microdialysis ethanol technique and 133Xe clearance. Clin Sci (Lond) 86:15–25
29. **Villanueva-Penacarrillo ML, Marquez L, Gonzalez N, Diaz-Miguel M, Valverde I** 2001 Effect of GLP-1 on lipid metabolism in human adipocytes. Horm Metab Res 33:73–77
30. **Baba AS, Harper JM, Buttery PJ** 2000 Effects of gastric inhibitory polypeptide, somatostatin and epidermal growth factor on lipogenesis in ovine adipose explants. Comp Biochem Physiol B Biochem Mol Biol 127:173–182
31. **Blaak EE, van Baak MA, Kempen KP, Saris WH** 1993 Role of α- and β-adrenoceptors in sympathetically mediated thermogenesis. Am J Physiol 264: E11–E17
32. **Hoeks J, van Baak MA, Hesselink MK, Hul GB, Vidal H, Saris WH, Schrauwen P** 2003 Effect of β1- and β2-adrenergic stimulation on energy expenditure, substrate oxidation, and UCP3 expression in humans. Am J Physiol Endocrinol Metab 285:E775–E782
33. **Balas B, Baig MR, Watson C, Dunning BE, Ligueros-Saylan M, Wang Y, He YL, Darland C, Holst JJ, Deacon CF, Cusi K, Mari A, Foley JE, DeFronzo RA** 2007 The dipeptidyl peptidase IV inhibitor vildagliptin suppresses endogenous glucose production and enhances islet function after single-dose administration in type 2 diabetic patients. J Clin Endocrinol Metab 92:1249–1255
34. **Morino K, Petersen KF, Dufour S, Befroy D, Frattini J, Shatzkes N, Neschen S, White MF, Bilz S, Sono S, Pypaert M, Shulman GI** 2005 Reduced mitochondrial density and increased IRS-1 serine phosphorylation in muscle of insulin-resistant offspring of type 2 diabetic parents. J Clin Invest 115:3587–3593
35. **Hulver MW, Berggren JR, Carper MJ, Miyazaki M, Ntambi JM, Hoffman EP, Thyfault JP, Stevens R, Dohm GL, Houmard JA, Muoio DM** 2005 Elevated stearoyl-CoA desaturase-1 expression in skeletal muscle contributes to abnormal fatty acid partitioning in obese humans. Cell Metab 2:251–261
36. **Marchand-Brustel Y, Gual P, Gremeaux T, Gonzalez T, Barres R, Tanti JF** 2003 Fatty acid-induced insulin resistance: role of insulin receptor substrate 1 serine phosphorylation in the retroregulation of insulin signalling. Biochem Soc Trans 31:1152–1156
37. **Vella A, Bock G, Giesler PD, Burton DB, Serra DB, Saylan ML, Dunning BE, Foley JE, Rizza RA, Camilleri M** 2007 Effects of dipeptidyl peptidase-4 inhibition on gastrointestinal function, meal appearance, and glucose metabolism in type 2 diabetes. Diabetes 56:1475–1480
38. **Scherbaum WA, Schweizer A, Mari A, Nilsson PM, Lalanne G, Jauffret S, Foley JE** 2008 Efficacy and tolerability of vildagliptin in drug-naive patients with type 2 diabetes and mild hyperglycaemia. Diabetes Obes Metab 10:675–682