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Hypertension and Type 2 Diabetes Are Associated With Decreased Inhibition of Dipeptidyl Peptidase-4 by Sitagliptin

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Context: Patients with diabetes often have comorbidities such as hypertension. It is not known how individual characteristics influence response to dipeptidyl peptidase-4 (DPP4) inhibitors.

Objective: We tested the hypothesis that individual characteristics, sitagliptin dose, and genetic variability in *DPP4* influence DPP4 activity during sitagliptin.

Design and Setting: *Post hoc* analysis of clinical and laboratory data from individuals randomized to sitagliptin versus placebo in crossover studies.

Patients and Interventions: Sixty-five subjects [27 with type 2 diabetes mellitus (T2DM) and hypertension, 38 healthy controls] were randomized to 100 mg/d sitagliptin or 200 mg sitagliptin and matching placebo in double-blind, crossover fashion. Fasting blood was obtained at baseline and 60 to 180 minutes after sitagliptin or placebo.

Main Outcome Measure(s): DPP4 activity and antigen during placebo and sitagliptin and DPP4 inhibition during sitagliptin.

Results: Sitagliptin 100 mg/d was less effective at inhibiting DPP4 activity in individuals with T2DM and hypertension than in healthy controls ($P = 0.001$, percent inhibition). In healthy controls, 100 mg/d sitagliptin was not as effective as single-dose 200 mg sitagliptin ($P = 0.001$, percent inhibition). *DPP4* genotypes rs2909451 TT ($P = 0.02$) and rs759717 CC ($P = 0.02$) were associated with DPP4 activity during sitagliptin. In multivariable analyses, T2DM with hypertension, sitagliptin dose, age, systolic blood pressure, DPP4 activity during placebo, and rs2909451 genotype were significantly associated with DPP4 activity during sitagliptin.

Conclusions: Sitagliptin is less effective in inhibiting DPP4 in individuals with T2DM and hypertension than in healthy controls. Higher doses of DPP4 inhibitors may be required in patients with the metabolic syndrome.

Freeform/Key Words: dipeptidyl peptidase-4 inhibition, DPP4, hypertension, metabolic syndrome, sitagliptin, type 2 diabetes mellitus

Abbreviations: BMI, body mass index; DBP, diastolic blood pressure; DPP4, dipeptidyl peptidase-4; FDA, Food and Drug Administration; HbA1C, hemoglobin A1C; HDL, high-density lipoprotein; SBP, systolic blood pressure; SNP, single nucleotide polymorphism; T2DM, type 2 diabetes mellitus.

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Dipeptidyl peptidase-4 (DPP4) is a ubiquitously expressed protease that cleaves the *N*-terminal of peptides containing a penultimate alanine or proline, including the incretins, glucagon-like peptide-1, and glucose-dependent insulinotropic polypeptide (or gastric inhibitory polypeptide) [1–3]. Inhibition of DPP4 decreases degradation of these hormones, leading to improved glycemic control through increased glucose-mediated insulin secretion, decreased glucagon release, and delayed gastric emptying [1, 2].

The DPP4 inhibitor sitagliptin was approved by the Food and Drug Administration (FDA) in 2006 for the treatment of type 2 diabetes mellitus (T2DM). The approved dose in patients with T2DM is 100 mg/d in patients with normal renal function [2]. In ongoing studies examining the cardiovascular effects of DPP4 inhibition in healthy subjects and individuals with T2DM and hypertension (NCT02130687, NCT01701973, NCT01413542), we have observed that the magnitude of DPP4 inhibition is less than previously reported [4–7]. In healthy subjects, doses of 50 to 200 mg all achieved >80% DPP4 inhibition [7]. We therefore examined the effect of individual characteristics, including age, gender, race, body mass index (BMI), and the presence or absence of T2DM and hypertension on DPP4 activity and inhibition by sitagliptin. In addition, we examined the effect of variants in the gene encoding DPP4 (*DPP4*) on inhibition by sitagliptin.

We hypothesized that individual characteristics, including genetic variability in *DPP4*, influence DPP4 activity and the extent of DPP4 inhibition during sitagliptin.

1. Methods

We studied healthy controls not taking any prescription medications and individuals with T2DM and hypertension who participated in one of three randomized, double-blind, placebo-controlled crossover studies in our laboratory and provided a DNA sample. All individuals were provided with and signed written informed consent, and the Vanderbilt Institutional Review Board reviewed and approved each study. Individuals were defined as having T2DM if they met one of three criteria, as follows: hemoglobin A1C (HbA1C) ≥6.5%, fasting plasma glucose ≥126 mg/dL, or plasma glucose ≥200 mg/dL after a 75 g oral glucose load. Diabetic subjects were further defined as hypertensive if they had one of the following: treatment with antihypertensive medication(s) for at least 6 months or, if not taking antihypertensive medication(s), had seated systolic blood pressure (SBP) ≥130 mm Hg documented on three occasions and diastolic blood pressure (DBP) ≥80 mm Hg documented on three occasions. Hypertension was defined as ≥130/80 mm Hg based on recommendations of the American Association of Clinical Endocrinologists (2015, 2016, and 2017 consensus statements) [8–10] and American Diabetes Association (2015) [11]. Baseline blood pressure and heart rate were measured using an automated oscillometric device (Dinamap; Critikon, Carlsbad, CA), and the mean of three repeated readings was calculated. Participants were randomized to receive either a single dose of 200 mg sitagliptin or matching placebo, or to receive repeated doses of 100 mg/d sitagliptin for 4 or 7 days or matching placebo. All subjects treated with 100 mg/d sitagliptin were analyzed as one group as maximal DPP4 inhibition was achieved after 4 days (DPP4 activity 8.88 ± 2.92 nmol/mL/min after 4 days and 9.69 ± 3.45 nmol/mL/min after 7 days, $P = 0.20$). Sitagliptin and placebo treatments were separated by a minimum of 1 week. Blood for measurement of DPP4 activity and antigen, glucose, and insulin was collected 60 to 180 minutes after the last dose of sitagliptin or placebo, after an overnight fast. We chose to collect DPP4 activity and antigen at these times as prior studies in healthy subjects have shown that, at time points 1 to 4 hours after 50 to 200 mg sitagliptin, there is at least 80% DPP4 inhibition from baseline [7]. Percent inhibition by sitagliptin was calculated as [1 − (DPP4 activity during sitagliptin/DPP4 activity during placebo)] × 100.

A. Laboratory Assays

DPP4 activity was performed by incubating 20 μL serum sample in 80 μL assay buffer (0.1 M Tris at a pH of 8.0; Bachem, Torrance, CA) for 30 minutes at 37°C with colorimetric substrate

(2 mM *l*-glycyl-*l*-prolyl *p*-nitroanilide hydrochloride; Sigma-Aldrich, St. Louis, MO) for a total reaction volume of 200 μL, as previously described [12, 13]. The enzyme activity was assessed by measuring the increase in specific absorbance at 405 nm at 0, 15, and 30 minutes and was expressed as nmol/mL/min. DPP4 antigen was performed by commercially available enzyme-linked immunosorbent assay (human CD26 platinum enzyme-linked immunosorbent assay kit; eBioscience, San Diego, CA). Plasma glucose was measured by the glucose oxidase method with a YSI glucose analyzer (YSI Life Sciences, Yellow Springs, OH). Insulin concentrations were measured by radioimmunoassay (EMD Millipore, Billerica, MA). The insulin assay cross-reacts with 38% intact human proinsulin, but not with C-peptide (≤0.01%). Lipids were measured via colorimetric enzymatic assay (reagents: Thermo-Fisher Infinity, Middletown, VA).

B. Genotyping Methods

Genomic DNA was extracted from whole blood using the AutoPure LS extraction system (Qiagen, Valencia, CA) by the Vanderbilt Technologies for Advanced Genomics (VANTAGE) core. Eleven *DPP4* variants were chosen for analyses: six variants that span the entire length of the *DPP4* gene, rs1014444, rs16822665, rs2909451, rs4664446, rs6733162, and rs7565794; four variants that were previously associated with DPPIV activity, rs2268894, rs2909443, rs741529, and rs759717 [14]; and one variant identified by our research group by a phenome-wide association study (PheWAS), rs116302758 (unpublished data). Sequenom MassARRAY iPlex genotyping (Agena Bioscience, San Diego, CA) system was used to genotype the six gene-spanning variants and four functional variants. The multiplexed assay was designed using the MassARRAY Assay Design Software (Agena Bioscience). The rs116302758 variant was genotyped using a TaqMan assay (Applied Biosystems, Foster City, CA). SDS v2.4 (Applied Biosystems) was used for the creation of cluster plots and the identification of sample-associated fluorescent markers for determination of genotype call.

C. Statistical Analyses

Results are presented as mean ± standard deviation, unless otherwise noted. Mann–Whitney U, Wilcoxon signed rank, and Spearman correlation were used to analyze continuous variables. One-way analysis of variance was used to analyze the relationship between *DPP4* genotype and DPP4 activity or antigen. Linear regression was used for multivariable analyses of DPP4 activity and percent inhibition during sitagliptin; variables initially included in the model were chosen based on univariate analyses and included sitagliptin dose, history of T2DM and hypertension, BMI, age, baseline mean SBP, baseline mean DBP, baseline fasting glucose, DPP4 genotype rs2909451, and DPP4 genotype rs749717, and variables were removed in a backward fashion. Using the method of Jones and Kenward [15], we tested for carryover and found no carryover effect of sitagliptin during crossover therapy. SPSS v23 software (Armonk, NY) was used for all statistical analyses. Values of $P \leq 0.05$ were considered significant.

2. Results

A. Relationship Between Individual Characteristics and DPP4 Activity and Antigen During Placebo

Table 1 provides the clinical characteristics of 65 subjects who had DNA and venous samples available for analyses. All participants with T2DM also had hypertension and met criteria for the metabolic syndrome as defined by the Adult Treatment Panel III criteria, with three or more of the following: waist circumference 102 cm or more in men and 88 cm or more in women, fasting triglycerides 150 mg/dL or higher, high-density lipoprotein (HDL) 40 mg/dL or lower in men and 50 mg/dL or lower in women, blood pressure 130/85 or higher, and fasting blood glucose of 100 mg/dL or higher or treatment of diabetes [16]. Twenty participants with T2DM and hypertension were taking metformin alone, whereas the other seven

Table 1. Subject Characteristics

Parameter	Normal Controls n = 38	Subjects With T2DM and Hypertension n = 27	Total n = 65
Age (years)	31.5 ± 10.6 (18.0–60.0)	57.0 ± 10.9 (33.1–75.6)	42.1 ± 16.5 (18.0–75.6)
Race (%)			
Black	4 (10.5)	9 (33.3)	13 (20.0)
Nonblack	34 (89.5)	18 (66.7)	52 (80.0)
White, non-Hispanic	31 (81.6)	16 (59.3)	47 (72.3)
White, Hispanic	2 (5.2)	2 (7.4)	4 (6.2)
Asian	1 (2.6)	0	1 (1.5)
Gender (%)			
Male	11 (28.9)	18 (66.7)	29 (44.6)
Female	27 (71.1)	9 (33.3)	36 (55.4)
Weight (kg)	67.7 ± 11.7 (49.0–101.0)	99.2 ± 18.9 (71.0–138.1)	80.8 ± 21.6 (49.0–138.1)
Body mass index (kg/m^2)	23.6 ± 2.3 (20.0–30.0)	33.4 ± 6.3 (25.6–49.5)	27.7 ± 6.5 (20.0–49.5)
SBP (mm Hg)	114.4 ± 9.7 (93.3–135.7)	137.0 ± 11.3 (121.3–162.0)	123.8 ± 15.2 (93.3–162.0)
DBP (mm Hg)	70.6 ± 7.1 (56.3–87.7)	82.5 ± 9.4(66.7–107.3)	75.5 ± 10.0 (56.3–107.3)
Heart rate (beats/min)	70.0 ± 10.4 (46.3–95.7)	70.1 ± 10.0 (52.0–88.3)	70.0 ± 10.1 (46.3–95.7)
Blood glucose (mg/dL)	84.1 ± 8.1 (70.0–112.0)	117.6 ± 25.8 (80.0–160.0)	98.0 ± 24.2 (70.0–160.0)
Fasting triglycerides (mg/dL)		128.4 ± 57.3 (50.0–253.0)	
Fasting HDL (mg/dL)		42.5 ± 10.1 (24.0–66.0)	
Waist circumference (cm)		111.1 ± 12.0 (90.5–133.0)	
Waist to hip ratio		0.97 ± 0.06 (0.85–1.07)	
Sitagliptin dose group (%)			
100 mg/d multiple doses	14 (36.8)	27 (100.0)	41 (63.1)
200 mg single dose	24 (63.2)	0	24 (36.9)

Mean ± standard deviation (range).

had diet-controlled T2DM. Subjects with T2DM and hypertension were taking ramipril (n = 7), valsartan (n = 9), amlodipine (n = 9), and hydrochlorothiazide (with n = 7) for blood pressure control; these medications were constant throughout the study. All participants with T2DM and hypertension received 100 mg/d sitagliptin. Healthy participants received either 100 mg/d sitagliptin or single dose 200 mg. There were no hypoglycemic events with either dose.

During placebo, DPP4 activity and antigen were significantly lower in individuals with T2DM and hypertension compared with in healthy controls (Table 2). During placebo, DPP4 activity decreased significantly with age and increasing BMI, as well as with increasing baseline SBP, DBP, and fasting glucose. DPP4 antigen also decreased with increasing BMI and increasing fasting blood glucose. DPP4 activity and antigen were similar during placebo in men and women and in blacks and whites.

B. Relationship Between Individual Characteristics and the Effect of Sitagliptin on DPP4 Activity

In healthy controls, a single dose of 200 mg sitagliptin reduced DPP4 activity to a greater extent than repeated doses of 100 mg/d sitagliptin. DPP4 activity was 5.83 ± 3.06 nmol/mL/min after 200 mg versus 8.88 ± 2.92 nmol/mL/min after repeated doses of 100 mg/d ($P = 0.005$). Percent inhibition was also greater after a 200 mg single dose of sitagliptin compared with repeated doses of 100 mg/d in healthy controls (Fig. 1). There was no effect of gender or race on DPP4 activity ($P = 0.49$ and $P = 0.92$, respectively) or percent inhibition ($P = 0.74$ and $P = 0.74$, respectively) following sitagliptin in healthy controls. As expected, neither dosing regimen of sitagliptin altered DPP4 antigen in healthy controls.

All subjects with T2DM and hypertension received repeated doses of 100 mg/d sitagliptin. The effects of sitagliptin on DPP4 activity and percent DPP4 inhibition were similar in men

Table 2. Relationship Between Subject Characteristics and DPP4 Variables During Placebo, n = 65

Subject Characteristics	DPP4 Activity (nmol/mL/min)	*P* Value	DPP4 Antigen (ng/mL)	*P* Value
Gender		NS		NS
Men	24.90 ± 6.92		427.98 ± 153.33	
Women	23.65 ± 5.81		442.71 ± 175.14	
Race		NS		NS
Black	21.51 ± 7.57		439.11 ± 191.20	
Nonblack	24.88 ± 5.85		435.39 ± 159.45	
T2DM and hypertension		0.002		0.05
No	26.26 ± 5.85		467.99 ± 150.35	
Yes	21.31 ± 5.87		391.30 ± 176.08	
Age	$r = -0.36$	0.004	$r = -0.13$	NS
BMI	$r = -0.28$	0.02	$r = -0.34$	0.005
Systolic blood pressure	$r = -0.28$	0.02	$r = -0.13$	NS
Diastolic blood pressure	$r = -0.36$	0.004	$r = -0.14$	NS
Fasting blood glucose	$r = -0.35$	0.004	$r = -0.28$	0.02

Mean ± standard deviation.
Abbreviation: NS, not significant.

and women ($P = 0.12$ and $P = 0.50$, respectively) and blacks and whites ($P = 0.96$ and $P = 0.08$, respectively) with T2DM and hypertension. A total of 100 mg/d sitagliptin inhibited DPP4 to a lesser extent in individuals with T2DM and hypertension than in healthy controls (Fig. 1). Sitagliptin did not affect DPP4 antigen in subjects with T2DM and hypertension.

In addition, percent DPP4 inhibition inversely correlated with BMI among all participants ($r = -0.56$, $P < 0.001$). This remained significant when separating by sitagliptin dose and T2DM and hypertension status [Fig. 2(A)]. There was also an inverse relationship between age and percent DPP4 inhibition among all individuals ($r = -0.31$, $P = 0.01$). Whereas during placebo, DPP4 activity decreased with increasing BMI, SBP, DBP, and fasting blood glucose, during sitagliptin, DPP4 activity increased significantly with increasing BMI ($r = 0.38$, $P = 0.002$), SBP ($r = 0.43$, $P < 0.001$), and DBP ($r = 0.30$, $P = 0.04$), and tended to increase with fasting blood glucose ($P = 0.08$). Percent DPP4 inhibition decreased with increasing SBP, DBP, and fasting blood glucose during sitagliptin in all participants [Fig. 2(B)–2(D)].

Among those with T2DM and hypertension in whom lipids were measured, increased fasting triglycerides ($r = 0.46$, $P = 0.02$) and lower HDL cholesterol ($r = -0.47$, $P = 0.01$) at

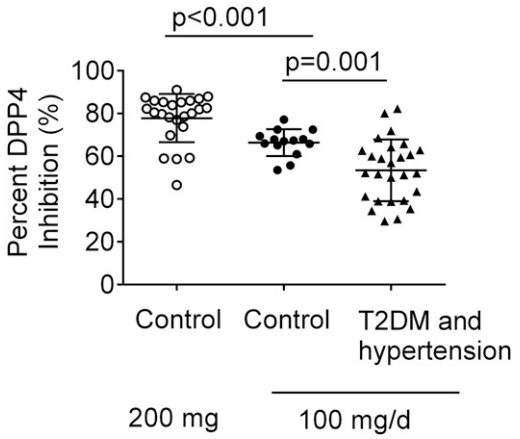

Figure 1. DPP4 inhibition by sitagliptin is decreased in individuals with T2DM and hypertension. Percent DPP4 inhibition in healthy controls on 200 mg sitagliptin single dose (n = 24, left), healthy controls on 100 mg/d sitagliptin for multiple doses (n = 14, middle), and individuals with T2DM and hypertension on 100 mg/d sitagliptin for multiple doses (n = 27, right). Mean ± standard deviation.

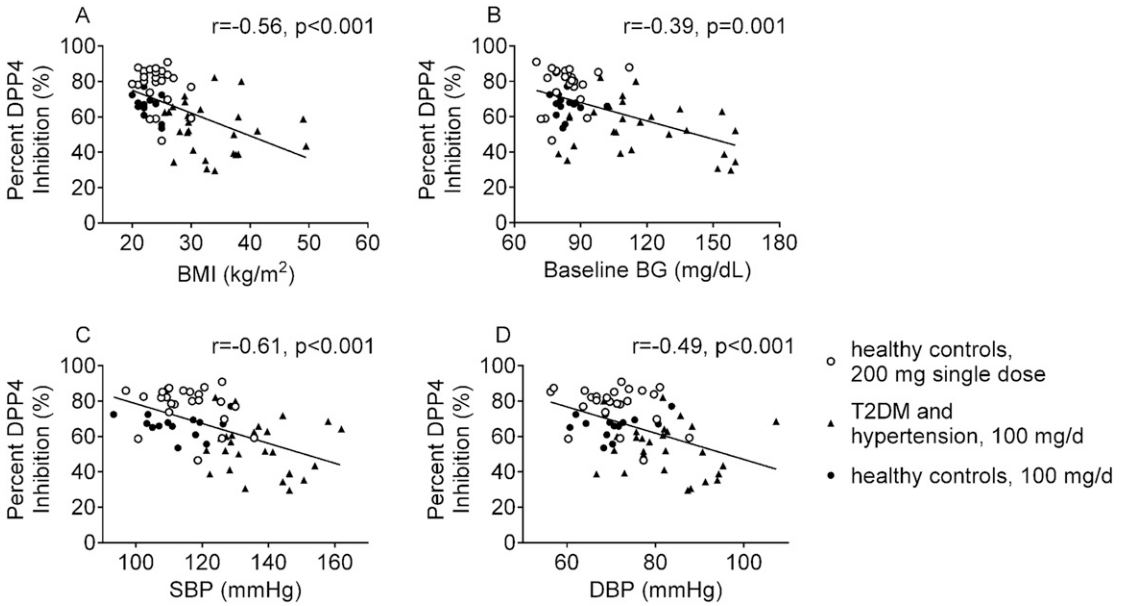

Figure 2. Percent DPP4 inhibition correlates inversely with BMI, fasting blood glucose, and blood pressure. Relationship between percent DPP4 inhibition and (A) BMI, (B) baseline blood glucose (BG), (C) baseline mean SBP, and (D) baseline mean DBP in healthy controls treated with 200 mg sitagliptin single dose (open circles), healthy controls treated with 100 mg/d sitagliptin for multiple doses (closed circles), and individuals with T2DM and hypertension treated with 100 mg/d sitagliptin for multiple doses (triangles).

baseline were significantly associated with higher DPP4 activity during sitagliptin, but were not associated with percent DPP4 inhibition ($P = 0.27$ and $P = 0.13$, respectively). Baseline fasting insulin was not associated with DPP4 activity during placebo or sitagliptin or with percent DPP4 inhibition.

C. Relationship Between DPP4 Genotypes and DPP4 Antigen and Activity

We next evaluated the effects of *DPP4* genotypes on DPP4 activity. All genotypes were in Hardy–Weinberg equilibrium (Supplemental Table 1). The linkage disequilibrium map for single nucleotide polymorphisms (SNPs) of interest is included as a Supplemental Fig. 1. One genotype was associated with lower DPP4 antigen concentrations during placebo treatment (rs4664446 AA: AG: GG = 417.8 ± 128.7: 491.6 ± 181.8: 367.9 ± 141.9 ng/mL, $P = 0.03$). This was not significant in a multivariable model.

Three genotypes were associated with higher DPP4 activity during placebo (rs6733162 GG) or during sitagliptin (rs2909451 TT and rs759717 CC) (Table 3; Fig. 3). The remaining genotypes were not significantly associated with DPP4 activity, antigen, or percent inhibition.

D. Multivariable Analyses

In a multivariable analysis, predictors of DPP4 activity during sitagliptin were sitagliptin dose, history of T2DM and hypertension, age, baseline SBP, rs2909451 genotype, and DPP4 activity during placebo (Table 4). Predictors of percent inhibition of DPP4 during sitagliptin were sitagliptin dose, history of T2DM and hypertension, age, and baseline SBP (Table 5).

3. Discussion

Sitagliptin is widely used to treat T2DM in the United States at the FDA-approved dose of 100 mg/d. We report that sitagliptin is less effective at inhibiting DPP4 in individuals with T2DM and hypertension than in healthy controls. In addition, contrary to previously published

Table 3. Relationship Between Genotypes and DPP4 Activity

DPP4 Activity (nmol/mL/min)	Major Allele	Genotypes Heterozygous	Minor Allele	*P* Value
rs2909451	CC (N = 45)	CT (N = 17)	TT (N = 3)	
Activity placebo	23.73 ± 6.79	24.54 ± 4.50	29.53 ± 7.07	NS
Activity sitagliptin	7.76 ± 3.55	7.99 ± 2.89	13.66 ± 4.97	0.02
rs6733162	CC (N = 26)	CG (N = 30)	GG (N = 9)	
Activity placebo	24.01 ± 6.62	22.84 ± 4.72	29.36 ± 8.02	0.02
Activity sitagliptin	8.51 ± 4.12	7.33 ± 3.03	9.43 ± 3.67	NS
rs759717	GG (N = 46)	GC (N = 16)	CC (N = 2)	
Activity placebo	24.00 ± 6.96	24.62 ± 4.63	26.11 ± 5.49	NS
Activity sitagliptin	7.83 ± 3.54	8.23 ± 2.80	15.01 ± 6.20	0.02

All other genotypes were not significant for DPP4 activity (rs1014444, rs4664446, rs16822665, rs7565794, rs2268894, rs2909443, rs741529, and rs116302758).
Abbreviation: NS, not significant.

literature, in healthy participants a single dose of 200 mg sitagliptin achieved greater percent DPP4 inhibition than repeated doses of 100 mg/d sitagliptin.

Initial studies of the pharmacokinetic and pharmacodynamics were conducted in healthy male subjects who were within 15% of their ideal body weight [7]. Pharmacokinetic studies in individuals with T2DM report percent DPP4 inhibition as 96% and 80.2% 2 hours after single doses of 200 mg and 25 mg, respectively, and 80.1% and 46.6% 24 hours after the same doses. Using modeling, the effect of 100 mg/d was extrapolated from this to coincide with 80% inhibition of DPP4 activity and 100 nM sitagliptin concentration [5]. In this prior study, patients with a BMI of 40 or greater were excluded and the mean BMI was 29.5 kg/m^2 [5], which is lower than in patients with T2DM and hypertension in the current study (33.4 kg/m^2 among T2DM with hypertension). Tatosian *et al.* studied overweight and obese individuals (mean BMI 32 kg/m^2) with T2DM treated with 100 mg/d sitagliptin for 5 days and reported that sitagliptin achieved >90% DPP4 inhibition after 2 days and maintained >90% DPP4 inhibition 24 hours after the fifth dose [4]. Herman *et al.* reported in nondiabetic obese individuals that the sitagliptin dose of up to 200 mg twice daily was well tolerated and achieved 90% DPP4 inhibition [6]. We report lower percent DPP4 inhibition in our subjects taking 100 mg/d sitagliptin than previously reported in the literature.

The impact of sitagliptin dose on metabolic endpoints has been studied in T2DM, but not in the context of percent DPP4 inhibition. Two studies have previously evaluated HbA1C, fasting insulin and blood glucose, and postprandial blood glucose in individuals with T2DM taking 200 mg/d sitagliptin versus 100 mg/d sitagliptin. The investigators did not find a significant difference between the effects of 200 mg/d versus 100 mg/d in the overall

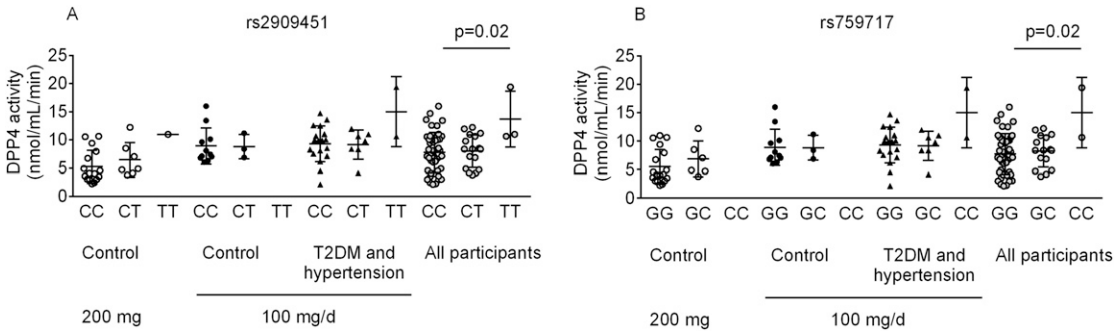

Figure 3. Relationship between rs2909451 and rs759717 genotype and DPP4 activity. *DPP4* genotype rs2909451 (A) and DPP4 activity and rs759717 (B) and DPP4 activity in the following: healthy controls treated with 200 mg sitagliptin single dose (n = 24, left), healthy controls treated with 100 mg/d sitagliptin multiple doses (n = 14, middle), individuals with T2DM and hypertension treated with 100 mg/d sitagliptin multiple doses (n = 27, right), and all participants (n = 65, far right). Mean ± standard deviation.

Table 4. Multivariable Analysis of Factors Associated With DPP4 Activity During Sitagliptin, All Individuals (n = 65)

Variable	Unstandardized B	Coefficients Standard Error	Standardized Coefficients, β	t	P Value
Model	−3.87	3.86		−1.00	0.32
Sitagliptin dose	−2.44	0.88	−0.33	−2.77	0.008
T2DM and hypertension	2.80	1.36	0.39	2.07	0.04
Baseline SBP	0.09	0.03	0.38	2.89	0.005
Age	−0.10	0.03	−0.44	−2.89	0.006
rs2909451	1.19	0.57	0.19	2.09	0.04
DPP4 activity during placebo	0.28	0.05	0.49	5.13	<0.001

Baseline DBP, BMI, and rs759717 were not significant and were removed from the model.

population on glycemic control, but in one of the studies 200 mg/d appeared to be more effective in lowering HbA1C in those patients with a pretreatment HbA1C <9% (similar to our inclusion criteria of HbA1C <8.7%) [17, 18]. These studies did not evaluate the effect of dosing on DPP4 activity after sitagliptin.

Differences in inclusion criteria may explain our observation that 100 mg/d sitagliptin does not fully inhibit DPP4 in T2DM and hypertension, in contrast to prior reports of subjects with T2DM. Specifically, an enrichment of patients with the metabolic syndrome may account for decreased DPP4 inhibition during sitagliptin, as all participants with T2DM and hypertension in this study met criteria for diagnosis of metabolic syndrome. We observed that SBP, DBP, and fasting glucose were associated with increased DPP4 activity and/or decreased DPP4 inhibition during sitagliptin. Increased fasting triglycerides and lower HDL levels were also associated with a higher DPP4 activity in participants with T2DM and hypertension in whom they were measured. Consistent with the hypothesis that components of the metabolic syndrome may influence DPP4 inhibition, Jamaluddin *et al.* reported that lower triglycerides and lower DBP are associated with a better therapeutic response to sitagliptin [19].

Prior studies have also suggested a relationship between insulin and blood glucose levels and DPP4 activity. Ryskjaer *et al.* reported that DPP4 activity increased with higher fasting insulin levels in T2DM [20]. We did not find this to be true among our subjects with T2DM and hypertension. Mannucci *et al.* reported that DPP4 activity was highest in poorly controlled T2DM (defined as HbA1C >8.5%) compared with those with newly diagnosed T2DM or without T2DM [21]. Our study excluded T2DM and hypertension participants with HbA1C >8.7%, which may contribute to the lack of signal for insulin levels in our participants with T2DM and hypertension. We did, however, find an inverse correlation between percent DPP4 inhibition by sitagliptin and fasting blood glucose.

It has been reported that DPP4 antigen expression and release are increased in isolated human adipocytes from both visceral adipose tissue and subcutaneous adipose tissue [22, 23]. Insulin has also been reported to increase the release of DPP4 antigen from human preadipocytes [24]. Others

Table 5. Multivariable Analysis of Factors Associated With Percent DPP4 Inhibition During Sitagliptin, All Individuals (n = 65)

Variable	Unstandardized B	Coefficients Standard Error	Standardized Coefficients, β	t	P Value
Model	100.66	16.12		6.25	<0.001
Sitagliptin dose	9.07	3.95	0.28	2.30	0.03
T2DM and hypertension	−13.56	6.12	−0.42	−2.22	0.03
Baseline SBP	−0.46	0.14	−0.44	−3.29	0.002
Age	0.37	0.15	0.39	2.55	0.01

Baseline DBP, BMI, DPP4 activity during placebo, and genotypes rs2909451 and rs759717 were not significant and were removed from the model.

have reported obesity and insulin resistance are associated with increased DPP4 antigen levels, but the impact of sitagliptin therapy on this was not established [25, 26]. Increased DPP4 expression or release could account for diminished inhibition during sitagliptin with increasing BMI. Paradoxically, however, we found instead that there was an inverse correlation between DPP4 antigen with BMI and fasting blood glucose during placebo.

We also tested the hypothesis that genetic variability in *DPP4*, the gene encoding DPP4, affects DPP4 activity during placebo and DPP4 inhibition after sitagliptin. The associations between DPP4 activity during sitagliptin and the SNPs rs2909451 and rs759717 have not been described previously in the literature. We found that the rs4664446 GG genotype (homozygous wild type) was associated with lower DPP4 antigen levels during placebo. Ahmed *et al.* reported that a different SNP variant, rs4664443 G>A, is associated with both diabetes and increased circulating DPP4 antigen concentrations in Malaysian individuals with T2DM [27]. They did not report testing for variants of rs4664446. Given these findings, further testing in a larger cohort to assess the associations between these genotypes and DPP4 related variables is warranted.

This study has several limitations. Because this was a *post hoc* analysis of several small studies assessing cardiovascular effects of sitagliptin, we did not measure the long-term effects of sitagliptin on glucose homeostasis in patients with T2DM and hypertension. We chose to collect DPP4 activity and antigen 1 to 3 hours after administration of sitagliptin based on previously published data showing that inhibition peaks 1 to 4 hours after dosing [7]. We did not, however, measure DPP4 activity serially over time or measure plasma sitagliptin concentrations. We also used a single DPP4 assay. We did not provide pharmacodynamics measurements other than fasting glucose. We did not have baseline oral glucose tolerance testing or HbA1C data for all healthy participants; however, diabetes was determined to be extremely unlikely based on medical history, normal fasting blood glucose levels, and lean BMI. Most of these individuals did not meet American Diabetes Association criteria for recommended screening for T2DM [28]. We did not test 200 mg/d among our participants with T2DM and hypertension, although this would have enabled additional comparisons. In addition, the sample size is too small to permit a rigorous assessment of genetic effects.

In conclusion, we report that the current FDA-approved dose of sitagliptin does not fully inhibit DPP4 in patients with T2DM and hypertension. Increased BMI and features of the metabolic syndrome may contribute to alterations in DPP4 antigen and activity in these patients. An implication of our findings is that individuals with T2DM and hypertension may benefit from sitagliptin doses >100 mg/d to achieve maximal therapeutic effectiveness.

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