

# Dipeptidyl Peptidase 4 Inhibition Increases Postprandial Norepinephrine via Substance P (NK1 Receptor) During RAAS Inhibition

Jessica R. Wilson,<sup>1,2,3</sup> Scott Jafarian Kerman,<sup>1</sup> Scott A. Hubers,<sup>1,4</sup> Chang Yu,<sup>5</sup> Hui Nian,<sup>5</sup> Eric Grouzmann,<sup>6</sup> Philippe J. Eugster,<sup>6</sup> Dustin S. Mayfield,<sup>1</sup> and Nancy J. Brown<sup>1</sup>

<sup>1</sup>Division of Clinical Pharmacology, Vanderbilt Department of Medicine, Vanderbilt University Medical Center, Nashville, Tennessee 37232; <sup>2</sup>Division of Endocrinology, Diabetes, and Metabolism, Vanderbilt Department of Medicine, Vanderbilt University Medical Center, Nashville, Tennessee 37232; <sup>3</sup>Division of Endocrinology, Diabetes and Metabolism, University of Pennsylvania Department of Medicine, Philadelphia, Pennsylvania 19104; <sup>4</sup>Department of Cardiovascular Medicine, Mayo Clinic, Rochester, Minnesota 55905; <sup>5</sup>Department of Biostatistics, Vanderbilt University Medical Center, Nashville, Tennessee 37232; and <sup>6</sup>Service de Pharmacologie Clinique, Laboratoire des Catecholamines et Peptides, University Hospital of Lausanne, 1005 Lausanne, Switzerland

**ORCID numbers:** 0000-0003-0402-5830 (J. R. Wilson); 0000-0003-0256-5251 (S. J. Kerman); 0000-0001-8129-2452 (S. A. Hubers); 0000-0002-3224-2536 (E. Grouzmann); 0000-0002-0197-6318 (P. J. Eugster); 0000-0001-7109-3142 (N. J. Brown).

**Context:** Dipeptidyl peptidase 4 (DPP4) inhibitors may increase the risk of heart failure. Decreased degradation of vasoactive peptides like substance P [also degraded by angiotensin-converting enzyme (ACE)] and Y1 agonists peptide YY (PYY 1-36) and neuropeptide Y (NPY 1-36) could contribute.

**Objective:** This study tested the hypothesis that there is an interactive effect of DPP4 inhibition and ACE inhibition (vs antihypertensive control subjects) on vasoactive peptides after a mixed meal.

**Participants and Design:** Fifty-three patients with type 2 diabetes and hypertension were randomized to double-blind treatment with ramipril, valsartan, or amlodipine for 15 weeks in parallel groups. During the 5th, 10th, and 15th weeks, participants also received placebo + placebo, sitagliptin 100 mg/d + placebo, and sitagliptin + aprepitant 80 mg/d in random order. On the last day of each crossover treatment, participants underwent a mixed-meal study.

**Results:** Sitagliptin increased postprandial glucagon-like peptide-1 and decreased glucose in all antihypertensive groups. Sitagliptin increased NPY 1-36 and decreased Y2 agonists NPY 3-36 and PYY 3-36 in all groups. During ramipril or valsartan, but not amlodipine, sitagliptin increased postprandial norepinephrine; substance P receptor blockade with aprepitant prevented this effect. Despite increased norepinephrine, sitagliptin decreased postprandial blood pressure during ACE inhibition.

**Conclusion:** DPP4 inhibition increases postprandial concentrations of the Y1 agonist NPY 1-36. During treatment with an ACE inhibitor or angiotensin receptor blocker, DPP4 inhibition increased postprandial norepinephrine through a substance P receptor-dependent mechanism. Increased NPY 1-36 and norepinephrine could increase risk of heart failure but did not result in higher postprandial blood pressure.

Abbreviations: ACE, angiotensin-converting enzyme; ARB, angiotensin receptor blocker; BNP, brain natriuretic peptide; DBP, diastolic blood pressure; DPP4, dipeptidyl peptidase 4; GLP-1, glucagon like peptide-1; MAP, mean arterial pressure; NK1, neurokinin receptor type 1; NPY, neuropeptide Y; PYY, peptide YY; RAAS, renin-angiotensin-aldosterone system; SBP, systolic blood pressure; T2DM, type 2 diabetes mellitus; VAS, visual analog scale.

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**Freeform/Key Words:** dipeptidyl peptidase-4 inhibition, DPP4, type 2 diabetes mellitus, hypertension, substance P, sitagliptin

Dipeptidyl peptidase-4 (DPP4) inhibitors are widely used in the treatment of patients with type 2 diabetes mellitus (T2DM). DPP4 is a transmembrane serine protease that selectively cleaves the amino terminal dipeptide from peptides with a penultimate proline or alanine, including the incretin hormones glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide [1]. DPP4 inhibitors improve glucose homeostasis by preventing degradation of incretins secreted after ingestion of a meal [2]. GLP-1 receptor activation increases postprandial insulin secretion, decreases glucagon release, and delays gastric emptying [2].

GLP-1 agonists generally have favorable cardiovascular outcomes in patients with T2DM and may reduce cardiovascular risk [3]. Even though DPP4 inhibitors increase endogenous GLP-1, they do not reduce cardiovascular disease and in some studies promote heart failure [4–6]. In addition to preventing the degradation of GLP-1, DPP4 inhibitors prevent the degradation of other vasoactive peptides, such as substance P, peptide YY (PYY 1-36), and neuropeptide Y (NPY 1-36) [1, 7]. Substance P is released from sensory C fibers and activates sympathetic nerve terminals via the neurokinin receptor type 1 (NK1) receptor, leading to release of norepinephrine and NPY 1-36 [8, 9]. PYY 1-36 is released by L cells of the intestine into plasma in response to a meal [10]. NPY 1-36 and PYY 1-36 activate the Y1 receptor, whereas their metabolites, via DPP4, NPY 3-36, and PYY 3-36, do not have activity at Y1 and activate Y2 and Y5 receptors [10–12]. Y1 receptor activation leads to vasoconstriction, potentiation of norepinephrine and alpha-adrenergic responses [13], and activation of cardiac fibroblasts [14]. In contrast, Y2 activation decreases sympathetic nerve terminal activation and norepinephrine release [11, 12] and suppresses appetite in animal models [10].

Over two-thirds of patients with T2DM also have hypertension, but the interactive effects of DPP4 inhibitors and antihypertensive medications on vasoactive peptides have not been extensively studied. Substance P is a shared substrate of DPP4 and angiotensin-converting enzyme (ACE). Combined ACE and DPP4 inhibition increases norepinephrine concentrations at baseline and in response to intra-arterial substance P infusion [15, 16]. In addition, DPP4 inhibition potentiates Y1 receptor-dependent vasoconstriction in response to intra-arterial NPY 1-36 infusion during interruption of the renin-angiotensin-aldosterone system (RAAS) by either ACE inhibition or angiotensin receptor blockade [17]. Increased substance P, NPY, and norepinephrine concentrations could contribute to an increased risk of heart failure [18, 19].

This study examined concentrations of Y1 agonists PYY 1-36 and NPY 1-36 and substance P receptor-dependent effects of DPP4 inhibition during concurrent antihypertensive therapy. Specifically, we compared the effect of crossover therapy with placebo, the DPP4 inhibitor sitagliptin, and sitagliptin given in combination with the substance P (NK1) receptor blocker aprepitant on hormone and hemodynamic responses to a mixed meal in patients with T2DM and hypertension who were first randomized to treatment with one of three antihypertensive agents: an ACE inhibitor, an angiotensin receptor blocker (ARB), or a calcium channel blocker. We hypothesized (i) that combined DPP4 and ACE inhibition would increase norepinephrine and NPY 1-36 in response to a mixed meal through a substance P-dependent mechanism (aim 1) and (ii) that DPP4 inhibition could potentiate the Y1-dependent effects of PYY 1-36 and NPY 1-36 after a mixed meal by decreasing their degradation through a substance P-independent mechanism (aim 2). We included ARB-treated patients to evaluate the possibility that DPP4 inhibition interacts with drugs that interrupt the RAAS, independent of ACE activity. We included patients treated with a calcium channel blocker to exclude the possibility of an interaction between DPP4 inhibition and antihypertensive therapy independent of the RAAS.

## 1. Research Design and Methods

### A. Subjects

The study was approved by the Vanderbilt Institutional Review Board and was conducted according to the Declaration of Helsinki. All volunteers provided written informed consent. Men and women 18 to 80 years old with T2DM and hypertension were eligible for enrollment. T2DM was defined by an untreated HbA1c  $\geq 6.5\%$  (48 mmol/mol) or greater, fasting plasma glucose  $\geq 126$  mg/dL (7.0 mmol/L), or 2-hour plasma glucose  $\geq 200$  mg/dL (11.1 mmol/L) following a 75-g oral glucose load or treatment with antidiabetic medication for at least 6 months. Hypertension was defined as a seated systolic blood pressure (SBP)  $\geq 130$  mm Hg or diastolic blood pressure (DBP)  $\geq 80$  mm Hg on at least three occasions or treatment with antihypertensive medications for at least 6 months, consistent with consensus statements on blood pressure control in individuals with diabetes at risk for cardiovascular disease [20]. Pregnancy was excluded in women of childbearing potential by  $\beta$ -human chorionic gonadotropin testing. Participants with poorly controlled diabetes [HbA1c  $> 8.7\%$  (72 mmol/mol)], long-term ( $> 12$  months) use of an antidiabetic medication other than metformin, use of insulin, type 1 diabetes, or secondary hypertension were excluded.

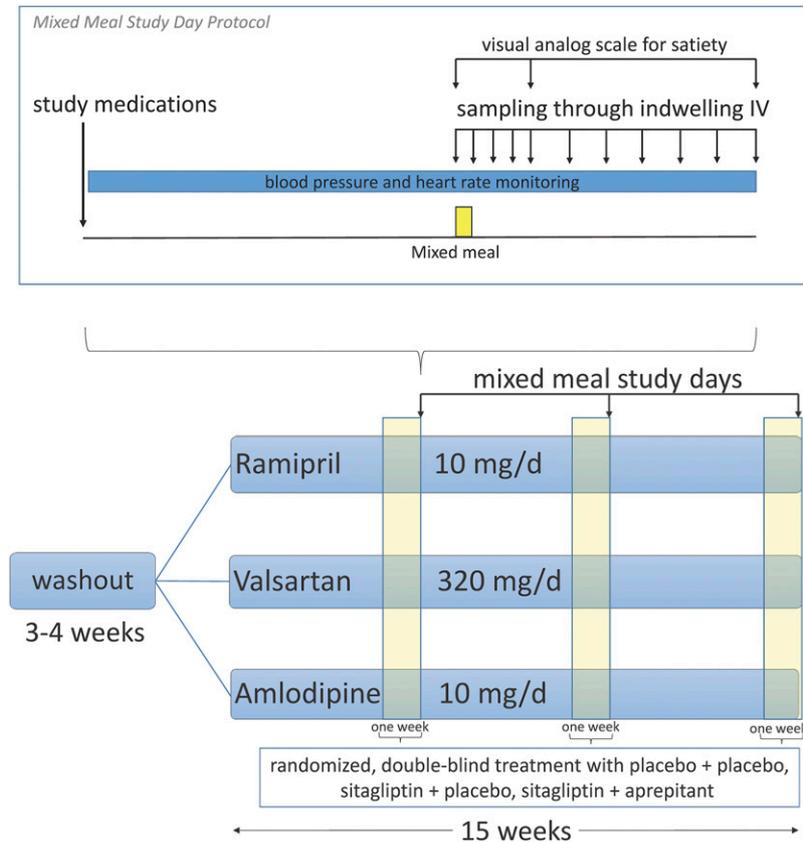
### B. Protocol

Participants taking antihypertensive and/or antidiabetic medications other than thiazide diuretics or metformin underwent washout from these other medicines for a minimum of 3 weeks (4 weeks for spironolactone). After washout, participants were randomized in a 1:1:1 ratio to ramipril, valsartan, or amlodipine at the following doses for 15 weeks: ramipril 5 mg/d for 3 days, then 10 mg/d; valsartan 160 mg/d for 3 days, then 320 mg/d; or amlodipine 5 mg/d for 3 days, then 10 mg/d (Fig. 1). After the first 4 weeks of antihypertensive treatment, subjects were randomized to the first of three 1-week crossover therapies: placebo/d + placebo/d, sitagliptin 100 mg/d + placebo/d, and sitagliptin 100 mg/d + aprepitant (125 mg, then 80 mg/d). Each crossover treatment was separated by 4 weeks to provide sufficient washout to avoid carryover effects (Fig. 1).

During the last day of each 1-week crossover treatment, subjects presented to the Vanderbilt Clinical Research Center after an overnight fast for a study day. Subjects were given their antihypertensive medication and their last dose of crossover medications between 7:00 and 8:00 AM. Blood pressure and heart rate were measured via an automated oscillometric device every 5 minutes during each study day (Dinamap; Critikon, Carlsbad, CA). Five hours after the last dose of study medication, subjects ingested a standardized mixed meal of 712 calories (45% fat, 33% carbohydrate, 22% protein) over 15 minutes. Blood samples were collected through an indwelling catheter before the meal and for 4 hours after the meal at time points 0, 15, 30, 45, 60, 90, 120, 150, 180, and 240 minutes. Before the meal and 60 and 240 minutes after, subjects completed a visual analog scale (VAS) to assess level of satiety and desire for a specific food type, as previously validated by Flint *et al.* [21].

### C. Laboratory Analyses

Plasma glucose was measured via glucose oxidase method using a glucose analyzer (YSI Life Sciences, Yellow Springs, OH) immediately after collection. All remaining samples were stored at  $-80^{\circ}\text{C}$  in aliquots until the time of assay. Plasma insulin samples were collected in tubes containing aprotinin, and insulin was measured by radioimmunoassay (EMD Millipore, Billerica, MA) [22]. The assay cross-reacts with 38% intact proinsulin but not with C-peptide ( $\leq 0.01\%$ ). Samples for GLP-1, PYY, and NPY were collected in tubes containing a cocktail of protease inhibitors to avoid *ex vivo* degradation of peptides (apstatin, vildagliptin, phenylmethylsulfonyl fluoride, EDTA, and phosphoramidon). Active GLP-1 was measured via Milliplex Human Metabolic Hormone Magnetic Bead Panel (EMD Millipore, Billerica, MA) and multiplex immunoassay (Meso Scale Discovery, Gaithersburg, MD). Total PYY was



**Figure 1.** Study protocol. After washout, subjects were randomized in a parallel design to receive 15 wk of antihypertensive treatment with one of three agents: ramipril, valsartan, or amlodipine. After 4 wk of treatment, subjects received the first of three 1-wk crossover interventions: placebo/d + placebo/d, sitagliptin 100 mg/d + placebo/d, and sitagliptin 100 mg/d + aprepitant 125 mg then 80 mg/d, in blinded, random order. Subjects underwent a mixed meal study day on the seventh and final day of crossover intervention. The three crossover interventions were separated by 4 wk to avoid carryover effects.

measured via radioimmunoassay, with 100% specificity to human PYY 1-36 and PYY 3-36, with sensitivity to 10 pg/mL (EMD Millipore, St. Charles, MO) [23]. PYY 3-36 was measured via radioimmunoassay, with 100% specificity for the 3-36 metabolite, no cross reactivity with PYY 1-36, and sensitivity to 20 pg/mL (EMD Millipore) [24]. PYY 1-36 was estimated as the difference between total PYY and PYY 3-36. NPY and its metabolites were measured via liquid chromatography–mass spectrometry with ultra–high-pressure liquid chromatography.

Plasma norepinephrine was measured via HPLC using electrochemical detection. Serum lipids were measured via colorimetric enzymatic assay (with reagents from Thermo-Fisher Infinity, Middletown, VA). Free fatty acids were measured via gas chromatography.

DPP4 activity was measured by incubating 20  $\mu$ L serum sample in 80  $\mu$ L assay buffer (0.1 M Tris at pH 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 volume of 200  $\mu$ L. DPP4 activity was assessed by measuring the increased specific absorbance at 405 nm at 0, 15, 30 minutes and was expressed as nmol/mL/min.

#### D. Statistical Analyses

The primary endpoints were norepinephrine and NPY (aim 1) and PYY (aim 2) levels. Secondary endpoints were blood pressure and heart rate. A sample size of 16 subjects per antihypertensive group was determined to provide 80% power to detect a difference in

postprandial norepinephrine concentrations between crossover treatments comparable to that observed in a prior study of a DPP4 inhibitor ( $280 \pm 89$  vs  $227 \pm 49$  pg/mL) [25]. This sample size provided >99% power to detect a difference in PYY 3-36 concentrations comparable to that previously reported during DPP4 inhibition [26]. The effect of DPP4 inhibition on postprandial endogenous NPY 1-36 concentrations has not been reported previously.

Results are presented as mean  $\pm$  SD unless otherwise noted. Although we have designed the study to avoid a carryover effect, we used a mixed-effects model for baseline measurements to test for potential first-order carryover effects. We found no evidence of carryover for any treatment on norepinephrine, NPY 1-36, NPY 3-36, PYY 1-36, GLP-1, insulin, SBP, DBP, mean arterial pressure (MAP), heart rate, triglycerides, or very-low-density lipoprotein. In the ramipril group only, there was evidence for carryover effect of sitagliptin + aprepitant on glucose. In the valsartan group only, there was evidence for a carryover effect of sitagliptin and sitagliptin + aprepitant on PYY 3-36 concentrations.

We used repeated measures ANOVA to evaluate the effect of crossover treatment (placebo + placebo, sitagliptin + placebo, sitagliptin + aprepitant) within each antihypertensive treatment group, followed by paired *t* tests between crossover treatments. For glucose in the ramipril group and PYY 3-36 in the valsartan group, we also fitted a mixed-effects model on crossover treatments with a carryover term included as a covariate. For blood pressure and heart rate, we conducted additional multivariable analysis using generalized least squares linear models with compound symmetry error covariance to control for sex and race. All hypotheses were tested at the level of  $\alpha = 0.05$ . We performed analyses using SPSS for Windows (Version 24.0, SPSS, Chicago) and the open source statistical package R (version 3.5.1, R Development Core Team, 2018).

## 2. Results

### A. Participant Characteristics

Fifty-three participants were randomized to antihypertensive therapy and received at least one of the randomized crossover treatments (Fig. 1, Supplemental Fig. 1 [27]). One participant in the ramipril treatment arm and one in the valsartan arm dropped out after the first crossover treatment, and these participants were not included in the analyses. Table 1 shows the characteristics of participants within each antihypertensive arm who completed all three crossover treatments.

### B. Effect of DPP4 Inhibition on Baseline (Preprandial) Characteristics

Table 2 provides the effect of crossover treatment on baseline characteristics (prior to mixed meal ingestion) within each antihypertensive treatment group. Sitagliptin increased fasting GLP-1 concentrations in all three antihypertensive groups. Sitagliptin did not affect fasting insulin concentrations. Sitagliptin significantly decreased fasting glucose in the valsartan and amlodipine groups. In the valsartan group, fasting glucose was significantly reduced only during sitagliptin and aprepitant, and fasting glucose was significantly lower during sitagliptin + aprepitant than during sitagliptin + placebo.

Treatment with sitagliptin with or without aprepitant significantly decreased fasting PYY 3-36 concentrations in all three antihypertensive treatment groups (Table 2). Sitagliptin increased fasting PYY 1-36 only in the valsartan group. Sitagliptin decreased fasting NPY 3-36 and increased fasting NPY 1-36 in all three antihypertensive treatment groups. Aprepitant did not affect NPY 1-36 or NPY 3-36.

Baseline norepinephrine concentrations were significantly higher during sitagliptin + placebo compared with placebo + placebo but not during sitagliptin + aprepitant in the ramipril treatment group. There was also a trend toward increased baseline norepinephrine during sitagliptin + placebo in the valsartan treatment group, but this did not reach statistical significance. There was no effect of crossover therapy on baseline MAP, SBP (not shown), DBP (not shown), or heart rate in any of the antihypertensive treatment groups.

**Table 1. Baseline Subject Characteristics Before Randomization**

Parameter	Ramipril (n = 15)	Valsartan (n = 16)	Amlodipine (n = 20)	All (n = 51)
Age, y	52.7 ± 5.9	56.5 ± 8.8	58.5 ± 13.1	56.2 ± 10.2
Sex, n (%)				
Male	9 (60.0)	10 (62.5)	11 (55.0)	30 (58.8)
Female	6 (40.0)	6 (37.5)	9 (45.0)	21 (41.2)
Race and ethnicity, n (%)				
Non-Hispanic white	12 (80.0)	11 (68.8)	10 (50.0)	33 (64.7)
Black	2 (13.3)	5 (31.3)	7 (35.0)	14 (27.5)
Hispanic white	0 (0)	0 (0)	2 (10.0)	2 (3.9)
Asian	1 (6.7)	0 (0)	1 (5.0)	2 (3.9)
Weight, kg	106.1 ± 20.0	96.9 ± 19.0	97.4 ± 16.6	99.8 ± 18.5
BMI, kg/m <sup>2</sup>	34.6 ± 5.3	32.5 ± 6.6	33.6 ± 5.8	33.5 ± 5.9
SBP, mm Hg	131.5 ± 11.4	135.0 ± 10.3	135.5 ± 11.4	134.2 ± 11.0
DBP, mm Hg	76.9 ± 5.9	82.5 ± 7.7	78.3 ± 7.7	79.2 ± 7.4
Heart rate, bpm	74.8 ± 10.6	71.0 ± 9.8	71.1 ± 10.4	72.2 ± 10.2
Fasting blood glucose, mg/dL	129.1 ± 27.5	119.4 ± 20.2	118.7 ± 28.8	122.0 ± 25.9
HbA1c, % (mmol/mol)	6.8 ± 1.0 (51 ± 12.6)	6.5 ± 0.8 (48 ± 14.75)	6.5 ± 0.7 (48 ± 15.84)	6.6 ± 0.8 (49 ± 14.75)
Triglycerides	165.1 ± 63.7	145.7 ± 57.5	111.9 ± 47.9	138.1 ± 59.3
HDL-cholesterol	43.2 ± 9.7	43.6 ± 12.3	43.9 ± 9.2	43.6 ± 10.2

Data are presented as means ± SD or number and percent.

Abbreviations: BMI, body mass index; HDL, high-density lipoprotein.

### C. Effect of DPP4 Inhibition on Metabolic and Hormone Responses to a Mixed Meal

GLP-1 concentrations increased significantly after the mixed meal in all three antihypertensive treatment groups (Fig. 2). Sitagliptin significantly increased GLP-1 concentrations further after the mixed meal in all treatment groups. Coadministration of the NK1/substance P receptor antagonist aprepitant blunted the GLP-1 response 60 minutes after the meal in the ramipril group. There was a significant but small effect of sitagliptin on postprandial insulin concentration in the ramipril and valsartan treatment groups. In the ramipril treatment group, aprepitant abolished this effect. Sitagliptin decreased glucose concentrations after the mixed meal in all three antihypertensive treatment groups. Coadministration of aprepitant slightly but significantly enhanced this effect at baseline in the valsartan treatment group and 3 to 4 hours after meal ingestion in the amlodipine group.

PYY 1-36 and PYY 3-36 increased after meal ingestion during placebo + placebo treatment in all three antihypertensive treatment groups (Fig. 3). There was no effect of sitagliptin or aprepitant on postprandial PYY 1-36 concentrations. Sitagliptin markedly decreased postprandial PYY 3-36 concentrations, and there was no effect of NK1/substance P receptor blockade.

Because we did not detect an effect of meal ingestion on concentrations of NPY 1-36 or its metabolites in a pilot study of nine participants (data not shown), we analyzed samples for NPY 1-36 and 3-36 at only two time points (*i.e.*, at baseline and 1 hour after meal ingestion). NPY 1-36 was significantly increased and NPY 3-36 significantly decreased during sitagliptin treatment, and concurrent treatment with aprepitant did not significantly affect this (Fig. 3).

Norepinephrine increased significantly 30 minutes after ingestion of the mixed meal during placebo treatment. Sitagliptin significantly accentuated the increase in norepinephrine in the ramipril and valsartan treatment groups, although the time-course of significance was slightly different (Fig. 4). Because norepinephrine concentrations were statistically similar in the patients treated with ramipril and valsartan during all crossover treatments, we also analyzed the effect of sitagliptin in the two RAAS inhibitor treatment groups combined. Sitagliptin significantly increased postprandial norepinephrine

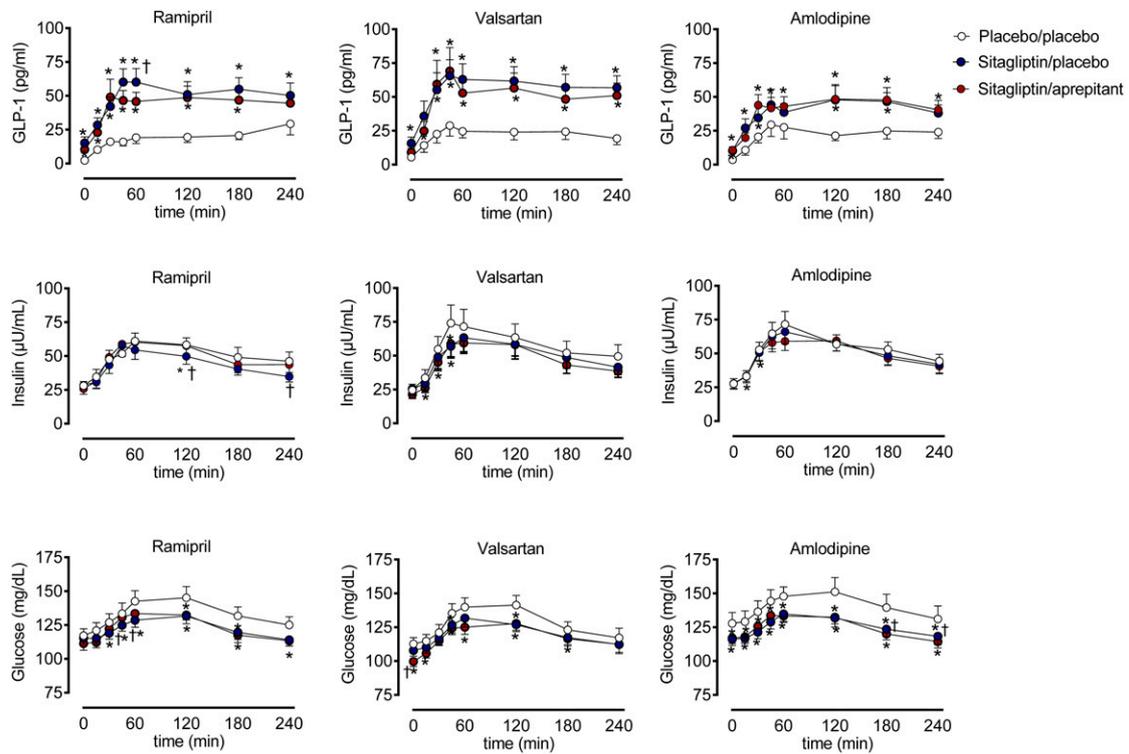
Table 2. Baseline Metabolic and Hemodynamic Parameters Before the Mixed Meal on Each Study Day

Variable	Ramipril			Valsartan			Amlodipine		
	Placebo/ Placebo	Sitagliptin/ Placebo	Sitagliptin/ Aprepitant	Placebo/ Placebo	Sitagliptin/ Placebo	Sitagliptin/ Aprepitant	Placebo/ Placebo	Sitagliptin/ Placebo	Sitagliptin/ Aprepitant
DPP4 activity, nmol/mL/min	22.2 ± 6.5	11.1 ± 7.8 <sup>a</sup>	9.0 ± 3.5 <sup>a</sup>	22.6 ± 7.0	9.9 ± 1.2 <sup>a</sup>	8.6 ± 3.5 <sup>a</sup>	21.4 ± 7.5	8.7 ± 2.7 <sup>a</sup>	7.4 ± 3.0 <sup>a</sup>
GLP-1, pg/mL	2.3 ± 2.1	15.1 ± 17.7 <sup>a</sup>	10.3 ± 11.5 <sup>a</sup>	5.4 ± 7.4	15.7 ± 18.4 <sup>a</sup>	9.0 ± 12.0	3.6 ± 4.1	10.2 ± 12.9 <sup>a</sup>	10.7 ± 8.5 <sup>a</sup>
Insulin, μU/mL	28.1 ± 12.0	28.4 ± 12.2	25.9 ± 15.9	24.6 ± 17.5	25.5 ± 18.0	21.4 ± 12.0	27.6 ± 17.1	27.7 ± 15.4	27.8 ± 17.8
Glucose, mg/dL	117.2 ± 19.9	115.3 ± 17.6	111.3 ± 19.4	112.7 ± 18.5	107.8 ± 16.8	99.7 ± 14.8 <sup>a,b</sup>	127.8 ± 36.3	116.2 ± 20.7 <sup>a</sup>	117.0 ± 18.2 <sup>a</sup>
PYY 1-36, pg/mL	86.9 ± 65.0	105.9 ± 52.5	85.9 ± 43.6	75.0 ± 26.4	102.2 ± 40.9 <sup>a</sup>	106.2 ± 48.5 <sup>a</sup>	85.7 ± 56.4	91.1 ± 37.9	99.2 ± 39.0
PYY 3-36, pg/mL	67.5 ± 31.0	39.8 ± 19.8 <sup>a</sup>	38.7 ± 20.2 <sup>a,c</sup>	74.1 ± 39.4	48.0 ± 33.8 <sup>a</sup>	48.8 ± 33.4 <sup>a</sup>	74.3 ± 50.2	42.3 ± 22.4 <sup>a</sup>	44.1 ± 24.9 <sup>a</sup>
NPY 1-36, pM	0.32 ± 0.32	0.54 ± 0.26 <sup>a</sup>	0.52 ± 0.29 <sup>a</sup>	0.27 ± 0.15	0.50 ± 0.26 <sup>a</sup>	0.47 ± 0.28 <sup>a</sup>	0.35 ± 0.28	0.52 ± 0.34 <sup>a</sup>	0.51 ± 0.33 <sup>a</sup>
NPY 3-36, pM	1.18 ± 0.80	0.39 ± 0.21 <sup>a</sup>	0.35 ± 0.09 <sup>a</sup>	1.08 ± 0.38	0.41 ± 0.21 <sup>a</sup>	0.37 ± 0.22 <sup>a</sup>	1.35 ± 1.02	0.40 ± 0.21 <sup>a</sup>	0.39 ± 0.22 <sup>a</sup>
Triglycerides, mg/dL	140.7 ± 57.8	150.7 ± 49.2	145.4 ± 65.0	122.1 ± 50.5	124.4 ± 51.7	114.5 ± 42.9	92.3 ± 34.6	97.3 ± 41.5	91.5 ± 30.5
FFA, μg/mL	215.2 ± 72.9	230.6 ± 68.3	217.4 ± 66.5	242.3 ± 68.7	256.0 ± 83.1	247.2 ± 81.4	263.7 ± 106.3	227.5 ± 79.7 <sup>a</sup>	240.6 ± 77.0
VLDL, g/dL	28.1 ± 11.6	30.2 ± 9.8	29.1 ± 13.0	23.3 ± 9.1	23.6 ± 9.3	21.9 ± 7.7	18.5 ± 7.1	19.5 ± 8.5	18.2 ± 6.2
NE, pg/mL	321.3 ± 132.6	363.9 ± 125.1 <sup>a</sup>	333.1 ± 167.4	384.4 ± 250.2	434.3 ± 212.3	356.5 ± 124.2	543.4 ± 228.4	502.4 ± 192.9	466.1 ± 135.5
MAP, mm Hg	94.2 ± 11.3	92.6 ± 10.7	91.0 ± 9.6	94.2 ± 11.6	96.1 ± 8.8	94.5 ± 8.6	96.0 ± 10.0	93.6 ± 8.9	92.9 ± 8.7
HR, bpm	69.9 ± 10.7	73.3 ± 9.2	72.0 ± 10.4	70.3 ± 11.9	71.9 ± 11.3	69.1 ± 8.6	71.8 ± 13.1	70.3 ± 11.6	71.7 ± 13.0

Data are shown as means ± SD.

Abbreviations: FFA, free fatty acids; HR, heart rate; NE, norepinephrine; VLDL, very-low-density lipoprotein.

<sup>a</sup>*P* < 0.05 vs placebo/placebo.<sup>b</sup>*P* < 0.05 vs sitagliptin + placebo.<sup>c</sup>*P* = not significant with adjustment for carryover.



**Figure 2.** Effect of 1-wk treatment with placebo + placebo (white circle), sitagliptin 100 mg/d + placebo (blue circle), and sitagliptin 100 mg/d + aprepitant 125 mg followed by 80 mg/d (red circle) on GLP-1, insulin, and glucose concentrations after ingestion of a mixed meal (time 0) in patients with type 2 diabetes and hypertension receiving ramipril 10 mg/d (n = 15), valsartan 360 mg/d (n = 16), or amlodipine 10 mg/d (n = 20). \* $P < 0.05$  vs placebo. † $P < 0.05$  vs sitagliptin + aprepitant.

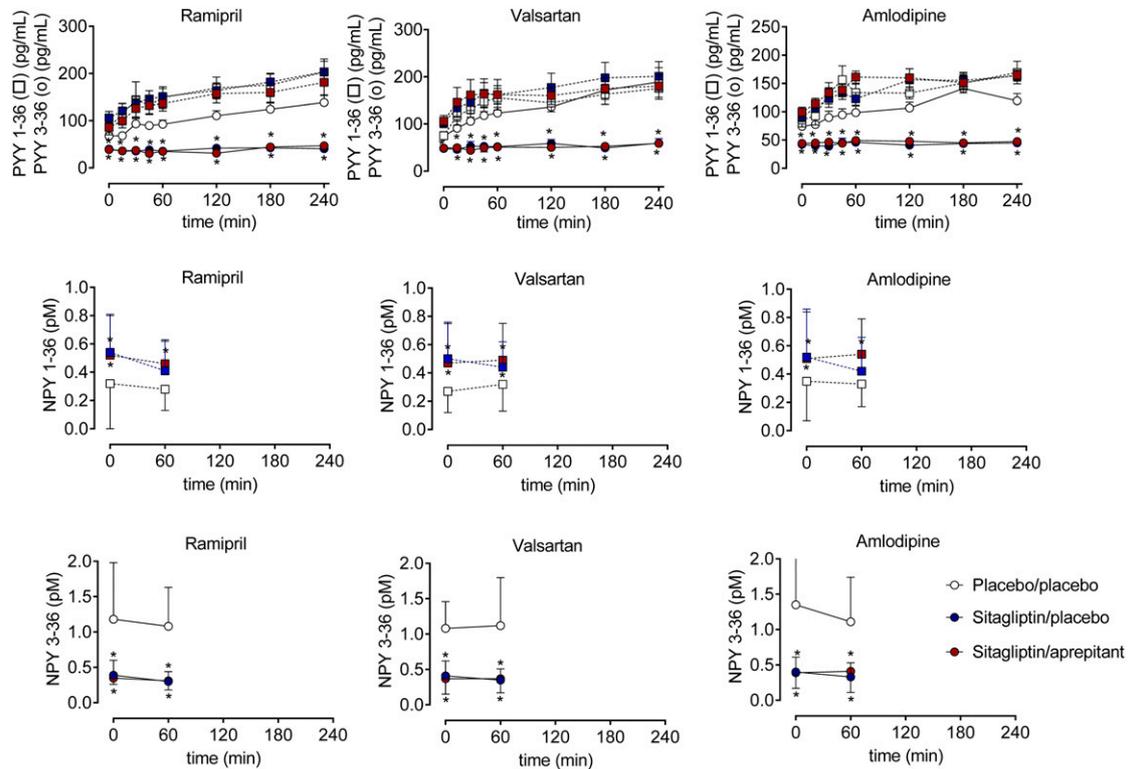
concentrations. Concurrent treatment with the NK1 receptor blocker aprepitant abolished this effect of sitagliptin on postprandial norepinephrine concentrations.

#### D. Effect of DPP4 Inhibition on Satiety After Mixed Meal Ingestion

Fullness increased and hunger decreased significantly 1 hour after the meal during all three crossover treatments in all three antihypertensive treatment groups (data not shown). The feeling of satisfaction increased significantly 1 hour after the meal in all three antihypertensive treatment groups. In the ramipril treatment group only, the increase in satisfaction was diminished during sitagliptin + placebo (VAS  $4.67 \pm 2.34$  at 1 hour) compared with during placebo + placebo (VAS  $5.85 \pm 2.03$ ,  $P = 0.008$ ) or sitagliptin + aprepitant (VAS  $5.85 \pm 2.42$ ,  $P = 0.037$ ). There was no other effect of sitagliptin or NK1 receptor blockade on fullness, hunger, or the feeling of satisfaction.

#### E. Effect of DPP4 Inhibition on Blood Pressure and Heart Rate After Mixed Meal Ingestion

Fasting blood pressure and heart rate were similar among treatment days. In the ramipril treatment group, postprandial MAP was significantly lower during sitagliptin + placebo and during sitagliptin + aprepitant compared with during placebo + placebo (Fig. 5). This reflected a significant effect of sitagliptin + placebo on SBP ( $P = 0.025$ ) and a significant effect of sitagliptin + aprepitant on both SBP ( $P = 0.020$ ) and DBP ( $P = 0.026$ ). There was no effect of sitagliptin alone on blood pressure in the valsartan or the amlodipine treatment group. In the amlodipine treatment group, sitagliptin + aprepitant decreased DBP ( $P = 0.003$  vs placebo + placebo and  $P = 0.015$  vs sitagliptin + placebo) and MAP ( $P = 0.007$  vs



**Figure 3.** Effect of 1-wk treatment with placebo + placebo (white), sitagliptin 100 mg/d + placebo (blue), and sitagliptin 100 mg/d + aprepitant 125 mg followed by 80 mg/d (red) on PYY 1-36 (square), PYY 3-36 (circle), NPY 1-36 (square), and NPY 3-36 (circle) in patients with type 2 diabetes and hypertension receiving ramipril 10 mg/d ( $n = 15$ ), valsartan 360 mg/d ( $n = 16$ ) or amlodipine 10 mg/d ( $n = 20$ ). \* $P < 0.05$  vs placebo. † $P < 0.05$  vs sitagliptin + aprepitant.

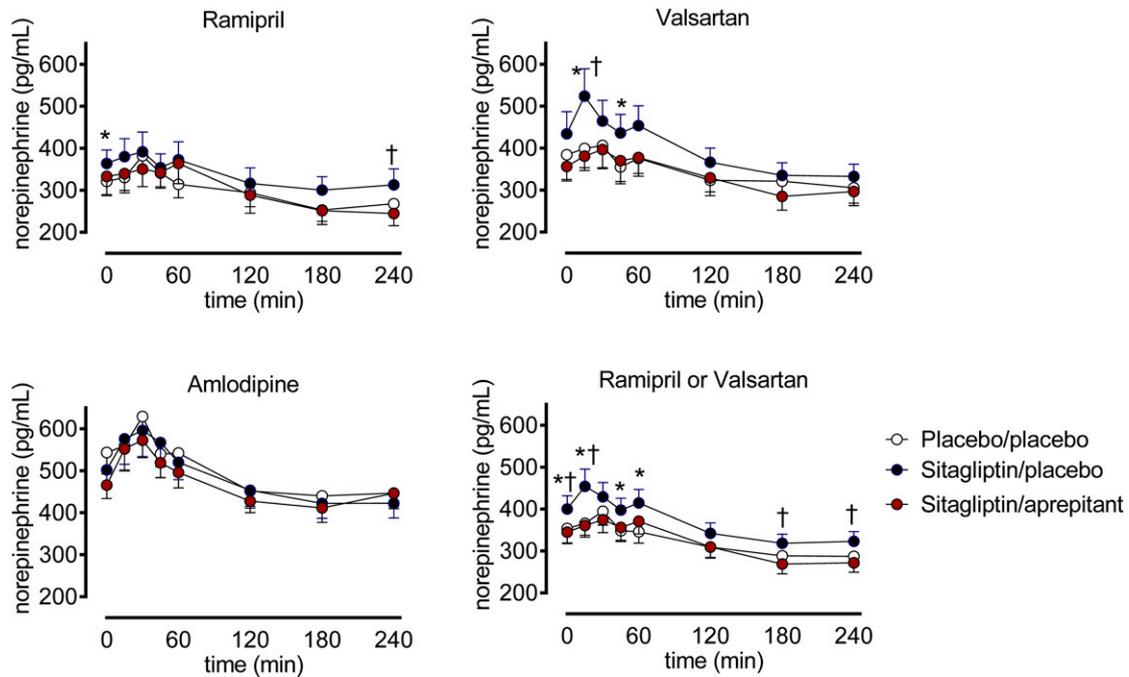
placebo + placebo and  $P = 0.054$  vs sitagliptin + placebo), suggesting an effect of aprepitant unrelated to DPP4 inhibition. There was no significant effect of sitagliptin + placebo on heart rate in any treatment group. Sitagliptin + aprepitant decreased heart rate vs sitagliptin + placebo in the valsartan treatment group and vs placebo + placebo in the amlodipine treatment group, again suggesting an effect unrelated to DPP4 inhibition. Similar results were observed after adjusting for race and sex.

#### F. Relationship Among Y1 and Y2 Agonist Concentrations, Norepinephrine, Blood Pressure, and Heart Rate

At 1 hour after meal ingestion, NPY 1-36 concentrations correlated significantly with norepinephrine during placebo + placebo ( $r = 0.370$ ,  $P = 0.008$ ), sitagliptin + placebo ( $r = 0.334$ ,  $P = 0.018$ ), and sitagliptin + aprepitant ( $r = 0.368$ ,  $P = 0.010$ ). NPY 1-36 correlated with PYY 1-36 during placebo + placebo ( $r = 0.374$ ,  $P = 0.007$ ) but not during either sitagliptin treatment.

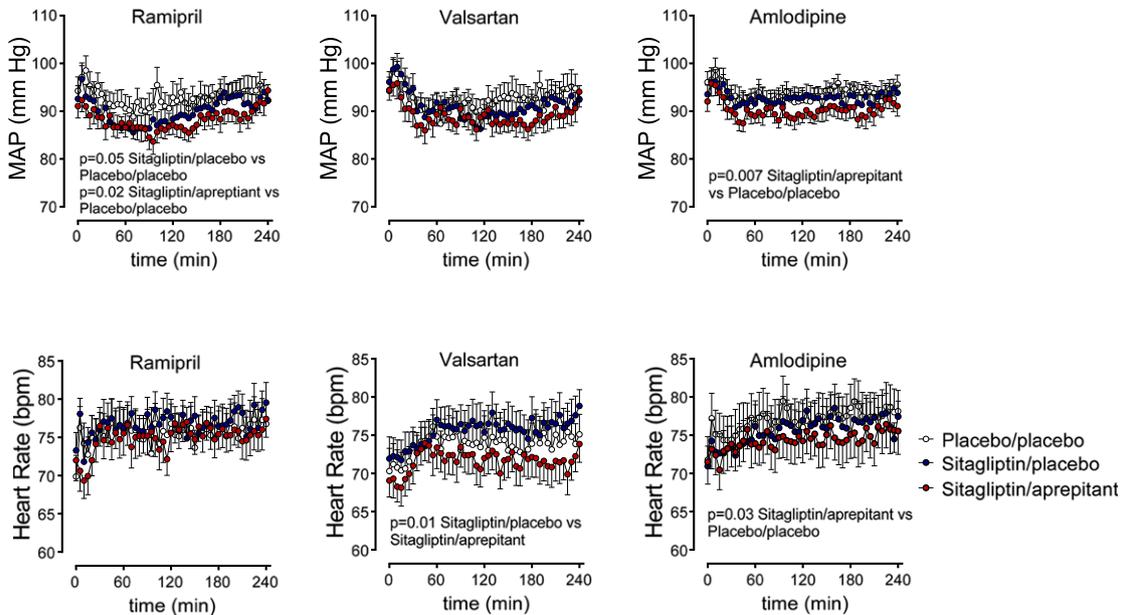
### 3. Discussion

This study examined Y1 agonist concentrations and substance P receptor–dependent effects of DPP4 inhibition in response to a meal during concurrent antihypertensive therapy. We report that sitagliptin increased circulating concentrations of the endogenous Y1 agonist NPY 1-36 and decreased circulating concentrations of the Y2 agonist NPY 3-36. The effects of DPP4 inhibition on Y1 and Y2 agonists did not involve endogenous substance P and were



**Figure 4.** Effect of 1-wk treatment with placebo + placebo (white circle), sitagliptin 100 mg/d + placebo (blue circle), and sitagliptin 100 mg/d + aprepitant 125 mg followed by 80 mg/d on plasma norepinephrine concentrations in patients with type 2 diabetes and hypertension receiving ramipril 10 mg/d (n = 15), valsartan 360 mg/d (n = 16), or amlodipine 10 mg/d (n = 20) \* $P < 0.05$  vs placebo. † $P < 0.05$  vs sitagliptin + aprepitant.

independent of concurrent antihypertensive therapy. In contrast, during treatment with either an ACE inhibitor or an ARB but not during treatment with a calcium channel blocker, DPP4 inhibition increased postprandial norepinephrine concentrations through a



**Figure 5.** Effect of 1-wk treatment with placebo + placebo (white circle), sitagliptin 100 mg/d + placebo (blue circle), and sitagliptin 100 mg/d + aprepitant 125 mg followed by 80 mg/d on MAP and heart rate in patients with type 2 diabetes and hypertension receiving ramipril 10 mg/d (n = 15), valsartan 360 mg/d (n = 16), or amlodipine 10 mg/d (n = 20). The  $P$  values provided are for comparison of the mean postprandial values among treatment arms.

substance P (NK1) receptor–dependent mechanism. Despite increased concentrations of the vasoconstrictor Y1 agonist NPY 1-36 and norepinephrine, there was no effect of sitagliptin on fasting blood pressure, and sitagliptin lowered postprandial blood pressure during ACE inhibition. Blockade of the substance P (NK1) receptor with aprepitant appeared to lower postprandial blood pressure and heart rate independently of DPP4 inhibition.

This study reports the effect of DPP4 inhibition on endogenous NPY 1-36 and NPY 3-36 concentrations after a meal. A few groups have reported the effect of DPP4 inhibition on responses to exogenous NPY 1-36 in adipocytes, where NPY 1-36 exerts antilipolytic effects [28, 29]. We observed no effect of meal ingestion on circulating NPY 1-36 or NPY 3-36. Previous studies measuring NPY using nonspecific immunoassays reported stable concentrations after meal ingestion [30] or a significant increase after ingestion after a meal with a high glycemic index but not after a meal with a low glycemic index [31], but immunoassays do not distinguish between NPY 1-36 and its metabolites. Interestingly, at 1 hour after the meal, circulating NPY 1-36 correlated with norepinephrine concentrations regardless of crossover treatment, consistent with colocalization of NPY 1-36 and norepinephrine in nerve terminals.

DPP4 inhibition with sitagliptin increased postprandial GLP-1 and decreased postprandial glucose in hypertensive patients with diabetes regardless of whether they were taking an ACE inhibitor, an ARB, or a calcium channel blocker. As reported previously [26, 32], DPP4 inhibition decreased formation of the Y2 agonist PYY 3-36 without altering postprandial PYY 1-36 concentrations, consistent with decreased PYY 1-36 secretion due to feedback inhibition [33]. Because PYY 3-36 reduces food intake [34], decreased PYY 3-36 may contribute to the lack of effect of DPP4 inhibition on weight. In support of this, we found an inverse relationship between PYY 3-36 and the desire to eat during placebo treatment but not during either sitagliptin treatment.

We observed a substantial effect of sitagliptin on postprandial norepinephrine concentrations in hypertensive patients with diabetes taking either an ACE inhibitor or an ARB but not in those taking amlodipine. We have previously reported an interactive effect of acute ACE inhibition and DPP4 inhibition on norepinephrine concentrations [15]. Boschmann *et al.* [25] reported higher norepinephrine concentrations in patients with diabetes, some of whom were taking antihypertensive agents other than beta-blockers, but the authors did not report what antihypertensive drugs the patients were taking. ACE and DPP4 share a common substrate, substance P, which stimulates norepinephrine release [8, 16]. The finding that cotreatment with the NK1/substance P receptor antagonist aprepitant abolished the increase in norepinephrine suggests that substance P mediates stimulation of the sympathetic nervous system. Importantly, aprepitant does not significantly affect gastric transit time [35]. The finding that norepinephrine was increased by sitagliptin during valsartan as well as during ramipril, however, indicates that the mechanism involves a pharmacodynamic interaction between substance P and the RAAS rather than a pharmacokinetic effect of ACE inhibition and DPP4 inhibition on substance P. Unfortunately, we were not able to measure endogenous substance P using a specific assay in this study.

Increased norepinephrine concentrations during ramipril and valsartan were not related to postprandial blood pressure. Norepinephrine was significantly higher at baseline (prior to meal ingestion) during sitagliptin (vs placebo) in the ramipril group when baseline blood pressure was not lower. In addition, postprandial norepinephrine was higher in both the ramipril and valsartan treatment groups during sitagliptin, whereas postprandial blood pressure was decreased only in the ramipril treatment arm. Despite the fact that concentrations of the vasoconstrictor NPY 1-36 were increased during sitagliptin and postprandial norepinephrine concentrations were higher during sitagliptin in patients randomized to either ramipril or valsartan, sitagliptin did not affect fasting blood pressure in any antihypertensive treatment group, and postprandial blood pressure was decreased during sitagliptin in patients taking the ACE inhibitor. This suggests the possibility that the vasoconstrictor effects of norepinephrine and NPY 1-36 are offset by an endogenous vasodilator in the postprandial period. In addition to stimulating the sympathetic nervous system, substance P causes vasodilation; nevertheless, the observation that blocking the substance P

(NK1) receptor tended to reduce blood pressure further during ramipril suggests that the sympatho-stimulatory effects of endogenous substance P predominate. ACE inhibitors potentiate bradykinin-mediated vasodilation, and bradykinin serves as a substrate for DPP4, but only after it has been cleaved by aminopeptidase P [1, 36]. Increased GLP-1 during sitagliptin may also have enhanced endothelium-dependent vasodilation during hyperinsulinemia following the mixed meal [37]. The vasodilator brain natriuretic peptide (BNP) is also a substrate of DPP4; however, BNP concentrations do not change in response to a meal [38], and DPP4 inhibition with sitagliptin does not alter the vasodilator effect of BNP [39]. In addition, the mechanisms for acute vs chronic blood pressure changes may be different. Jackson *et al.* [40] and others [41] have shown that, during acute DPP4 inhibition in animal models and *ex vivo*, changes in blood pressure are mediated by DPP4 substrates on the vasculature to affect peripheral vascular resistance. During chronic DPP4 inhibition with sitagliptin, however, DPP4 substrate activity at the level of the kidney and renovascular responsiveness/tone may contribute to increases in blood pressure seen in hypertensive animals during DPP4 inhibition compared with baseline and vs normotensive animals [40]. Similarly, in humans, acute vs chronic changes in NPY and renovascular tone may also contribute.

Regardless of the effects on blood pressure, increased NPY 1-36 and substance P-dependent increases in norepinephrine during inhibition of the RAAS could contribute to a lack of cardiovascular benefit of treatment with DPP4 inhibitors compared with GLP-1 agonists [42] as well as to increased risk of heart failure in some clinical trials of DPP4 inhibitors. Immunoreactive NPY and norepinephrine are increased during heart failure exacerbations and are associated with poor outcomes in patients with heart failure [19, 43, 44]. Although large cardiovascular outcomes studies of DPP4 inhibitors did not report neuropeptide or norepinephrine concentrations, it is possible that these may have been higher during DPP4 inhibition with saxagliptin (SAVOR-TIMI 53) and those without prior history of heart failure during alogliptin (EXAMINE) to contribute to the heart failure signal in these subjects [5, 6]. However, >70% of patients took an ACE inhibitor or ARB during the cardiovascular outcomes trials, thus limiting potential comparison of RAAS therapies to alternative antihypertensive therapies (such as calcium channel blockade, as we were able to in the current study), and there may have been confounding due to concurrent medication use, such as beta blockade [4, 6, 45].

In summary, DPP4 inhibition shifts the relationship between Y1 agonists and Y2 agonists after a meal. DPP4 inhibition increased NPY 1-36 concentrations through a substance P receptor-independent mechanism, specifically through decreased degradation. In contrast, during inhibition of the RAAS by either an ACE inhibitor or an ARB, DPP4 inhibition increased postprandial norepinephrine through a substance P-dependent mechanism. Future studies are needed to determine the long-term consequences of increases in Y1 agonists and norepinephrine.

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the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of data analysis.

## Additional Information

**Correspondence:** Nancy J. Brown, MD, Vanderbilt Department of Medicine, Division of Clinical Pharmacology, Vanderbilt University Medical Center, D-3100 Medical Center North, Nashville, Tennessee 37232-2358. E-mail: [nancy.j.brown@VUMC.org](mailto:nancy.j.brown@VUMC.org).

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**Data Availability:** All data generated or analyzed during this study are included in this published article or in the data repositories listed in the references.

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