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Acute effects on glucose tolerance by neprilysin inhibition in patients with type 2 diabetes

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Abstract

Aims: Sacubitril/valsartan is a neprilysin-inhibitor/angiotensin II receptor blocker used for the treatment of heart failure. Recently, a post-hoc analysis of a 3-year randomized controlled trial showed improved glycaemic control with sacubitril/valsartan in patients with heart failure and type 2 diabetes. We previously reported that sacubitril/valsartan combined with a dipeptidyl peptidase-4 inhibitor increases active glucagon-like peptide-1 (GLP-1) in healthy individuals. We now hypothesized that administration of sacubitril/valsartan with or without a dipeptidyl peptidase-4 inhibitor in patients with type 2 diabetes via increased active GLP-1.

Methods: We performed a crossover trial in 12 patients with obesity and type 2 diabetes. A mixed meal was ingested following five respective interventions: (a) a single dose of sacubitril/valsartan; (b) sitagliptin; (c) sacubitril/valsartan + sitagliptin;

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes. © 2022 The Authors. *Diabetes, Obesity and Metabolism* published by John Wiley & Sons Ltd. (d) control (no treatment); and (e) valsartan alone. Glucose, gut and pancreatic hormone responses were measured.

Results: Postprandial plasma glucose increased by 57% (incremental area under the curve 0-240 min) (p = .0003) and increased peak plasma glucose by 1.7 mM (95% Cl: 0.6-2.9) (p = .003) after sacubitril/valsartan compared with control, whereas postprandial glucose levels did not change significantly after sacubitril/valsartan + sitagliptin. Glucagon, GLP-1 and C-peptide concentrations increased after sacubitril/valsartan, but insulin and glucose-dependent insulinotropic polypeptide did not change.

Conclusions: The glucose-lowering effects of long-term sacubitril/valsartan treatment reported in patients with heart failure and type 2 diabetes may not depend on changes in entero-pancreatic hormones. Neprilysin inhibition results in hyperglucagonaemia and this may explain the worsen glucose tolerance observed in this study. ClinicalTrials.gov (NCT03893526).

KEYWORDS

clinical trial, drug mechanism, GLP-1, glucagon, glycaemic control

1 | INTRODUCTION

Sacubitril/valsartan, a combined neprilysin-inhibitor/angiotensin II receptor blocker used for treatment of chronic heart failure,¹ was recently reported to improve glycaemic control [glycated haemoglobin (HbA1c)] in patients with type 2 diabetes and heart failure in a post hoc analysis of a randomized controlled trial.² This gave rise to several theories regarding the mechanism behind the effects of sacubitril/valsartan on glucose control and metabolism in general.³ One prevailing hypothesis is that sacubitril/valsartan improves glucose control and metabolism by increasing plasma concentrations of the incretin hormone glucagon-like peptide-1 (GLP-1).⁴ Drugs that increase levels of endogenous [i.e. dipeptidy] peptidase-4 (DPP-4) inhibitors] or exogenous GLP-1 (i.e. GLP-1 receptor agonists) are widely used in the treatment of type 2 diabetes. Sacubitril is a neprilysin inhibitor, which prevents the cleavage of a number of vasoactive peptides⁵ including GLP-1.⁶ We previously reported that sacubitril/valsartan increases plasma concentrations of active GLP-1 when combined with a DPP-4 inhibitor in healthy individuals,⁷ supporting the notion that GLP-1 may contribute to the reported improvement in glucose control in patients with type 2 diabetes and heart failure.⁴

The aim of this study was to investigate the acute effect on postprandial glucose concentrations and entero-pancreatic hormone responses in patients with type 2 diabetes when treated with sacubitril/valsartan, the DPP-4 inhibitor sitagliptin, both agents combined, or no treatment (control).

2 | RESEARCH DESIGN AND METHODS

Before initiation of the study, ethical approval was obtained from the Regional Ethics Committee of the Capital Region of Denmark (H-

18000360, 66 893) and the Danish Data Protection Agency (2012-58-0004). The study was registered at ClinicalTrials.gov (NCT03893526) and conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants before inclusion. Twelve male participants with type 2 diabetes were recruited. Participants were eligible if they were diagnosed with type 2 diabetes according to the criteria of the World Health Organization, i.e. plasma glucose concentrations >7.0 mmol/L after an overnight fast. Only patients on oral antidiabetic agents were included: metformin treatment alone (10 patients), metformin combined with a DPP-4 inhibitor (one) or an sodium-glucose cotransporter 2 inhibitor alone (one). All glucose-lowering drugs were paused for a minimum of 3 days before each experimental day. Other drugs were evaluated individually and paused 1-2 days to avoid interference with study medications (see Table S1 for full list of medications). Inclusion criteria were age 30-70 years, body mass index >25 kg/m² and weight stability (±3 kg) for at least 3 months before screening. Exclusion criteria included acute sickness in the 14 days before screening, alcohol or drug abuse, a diagnosis of cancer, heart, liver, kidney or respiratory diseases and blood donation within 3 months before screening.

2.1 | Study design

The study was a crossover clinical trial with five visits: (1) single dose of a neprilysin-inhibitor/angiotensin II receptor blocker, 194 mg sacubitril/206 mg valsartan (Novartis Pharma GmbH, Nürnberg, Germany), 60 min before the meal; (2) DPP-4 inhibitor, sitagliptin 100 mg [Merck Sharp & Dohme (Italia) S.p.A., Pavia, Italy] the night before as well as 120 min before the meal; (3) single dose of 194 mg sacubitril/206 mg valsartan and two doses of sitagliptin (similar as in interventions 1 and 2); (4) no treatment (control); and (5) a single dose of valsartan (200 mg; KRKA Sverige AB, Stockholm/Tukholma) 60 min before the meal. All participants completed interventions 1-4, but only seven (of the 12) participants were available for the valsartan alone day (intervention 5), which was because valsartan alone day first was added later and therefore was not included for the first five study participants. Data from the valsartan day are therefore shown in the supplementary data file. Randomization of the experimental days was performed at screening by drawing a number from an envelope, with each number representing a fixed order of the test days.

2.2 | Study days

Study participants were instructed to fast overnight for at least 12 h, to be sedentary for 12 h before experiments and to use passive transportation on the experimental days. On each study day, participants were placed in a hospital bed, a catheter was inserted into a forearm vein and four blood samples were drawn (-60, -30, -15, 0), with t = -60 min taken as baseline. At t = 0 min a standardized solid meal (34E% carbohydrates, 45E% fat, 21E% protein, total caloric content of 487 kcal) was served and consumed evenly over 10 min. Blood was sampled frequently after the meal (at t = 15, 30, 45, 60, 90, 120, 150, 180, 210 and 240 min). Blood pressure and heart rate were also measured at these time points.

2.3 | Sample collection and biochemical analyses

Blood for the analysis of plasma glucose was collected in EDTA-Eppendorf tubes, which were centrifuged immediately, and plasma was analysed at the bedside using YSI model 2300 STAT Plus (YSI, Yellow Springs, OH, USA).

Prechilled EDTA-tubes containing the addition of a DPP-4 inhibitor (valine-pyrrolidide, final concentration of 0.01 M) were used for the collection of samples for measurements of total⁸ and intact GLP-1,⁹ total¹⁰ and intact glucose-dependent insulinotropic polypeptide (GIP),¹¹ and glucagon.¹² Serum was prepared using clot-activator tubes for measurements of neprilysin activity, insulin, C-peptide, fatty acids and triglycerides. Neprilysin activity was measured using a previously described in-house assay.¹³ Insulin, C-peptide, fatty acids and triglycerides were analysed using Roche COBAS 8000 system. Clot activator tubes were left at room temperature to coagulate for 30 min, whereas EDTA-tubes were placed on ice immediately until centrifuged at 4°C (2000 g for 10 min). Samples were frozen and stored at -80°C until analysis.

2.4 | Analysis of degradation of C-peptide by neprilysin

In vitro cleavage activity was assayed as reported previously¹³ by adding 20 ng of neprilysin (R&D Systems, Abingdon, NB) to 2 μ g of atrial natriuretic peptide (ANP; SynPeptide, Shanghai, China) or C-peptide (Schafer-N, Copenhagen, Denmark) in a total volume of 20 μ l. Reactions were performed in 50 mM Tris, 0.05% Brij-35, pH 9 and incubated at 37°C. Product development was monitored after 15 min and 60 min by MALDI-TOF analysis.

2.5 | Statistical considerations

The primary outcome was the difference in postprandial glucose peak between sacubitril/valsartan + sitagliptin compared with control. Sample size was calculated using $\alpha = 0.05$, a variance of 5%⁷ and a power of 90% to detect an estimated difference of 1 mmol/L using a mixed effect statistical model.

Area under the curve (AUC) was calculated using the trapezoidal rule. Incremental AUC (iAUC) was calculated as the baseline corrected positive peak area during the 240 min period after the meal (iAUC_{0-240 min}). A one-way ANOVA, with post hoc correction for multiple comparison (Sidak), was used to compare differences in fasting and peak concentrations as well as the AUC across interventions. A mixed effect model was applied to test the effect of treatment and time. Additive and synergistic effects were estimated by factorial analysis. Calculations and illustrations were made using GraphPad Prism version 8.0.2 for windows (GraphPad software, La Jolla, CA, USA) and STATA 17 (StataCorp, College Station, TX, USA). Data are presented as means ± SEM unless otherwise indicated.

TABLE 1 Data are presented as means (95% CI)

	$\begin{array}{l} \text{Participants} \\ \text{(n}=\text{12)} \end{array}$		
Gender	Male		
Age (years)	63 (60-66)		
Duration of diabetes (years)	7 (5-9)		
Weight, (kg)	97 (88-106)		
Body mass index kg/m ²	31 (28-33)		
HbA1c (%)	7 (6.5-7.3)		
HbA1c (mmol/mol)	51 (47-56)		
Plasma glucose at screening (mmol/L)	8.3 (7.6-8.9)		
Fasting plasma glucose, on the experimental day (mmol/L)	8.7 (7.8-9.6)		
Systolic blood pressure (mmHg)	138 (131-146)		
Diastolic blood pressure (mmHg)	81 (73-88)		
Alanine aminotransferase (U/L)	36 (27-46)		
Total cholesterol (mmol/L)	4.3 (3.5-5.0)		
Low-density lipoproteins (mmol/L)	2.2 (1.7-2.8)		
Triglycerides (mmol/L)	2.2 (1.4-3.1)		

Abbreviation: HbA1c, glycated haemoglobin.

3 | RESULTS

3.1 | Patient characteristics

Anthropometric and standard laboratory characteristics of study participants are shown in Table 1. Study participants had an average diabetes duration of 7 years, their average HbA1c was 7%, and they were obese (body mass index: 31 kg/m^2). All participants received glucose-lowering therapy. Eleven of 12 participants were treated with metformin alone (10 participants) or in combination with a DPP4 inhibitor (one), and one participant received sodium-glucose cotransporter 2 inhibitor monotherapy (Table S1). None received insulin therapy. None of the patients had heart failure nor treated with anticongestive medications.

3.2 | Glucose tolerance (primary outcome)

Mean fasting plasma glucose on the control day was 8.8 mmol/L and did not differ significantly (p > .3) between the experimental days (Table 2).

Sacubitril/valsartan administered alone significantly increased postprandial plasma glucose concentrations by 57% (iAUC_{0-240 min}) (p = .0003) and increased peak plasma glucose by 1.7 mM (95% CI: 0.6-2.9) (p = .003) compared with the control day (Figure 1A,B and Table 2). Sacubitril/valsartan combined with sitagliptin had no significant effect on postprandial plasma glucose (iAUC or peak) compared with the control day (p > .70) or sitagliptin alone (p > .5) (Figure 1A,B, Table 2).

Sitagliptin and valsartan monotherapies had no effect on postprandial plasma glucose concentrations (iAUC or peak) compared with the control day (p > .9) (Table 2, Figure S2).

3.3 | Beta cell function

3.3.1 | Insulin

Serum insulin concentrations at fasting were not significantly different across study days (p > .3).

TABLE 2 Plasma glucose and entero-pancreatic hormone concentrations in the fasting state before compound ingestion and during a standardized mixed-meal test 0-240 min, investigated during four different treatments with either control, sacubitril/valsartan, sitagliptin, sacubitril/valsartan plus sitagliptin, or valsartan

	Control	Sacubitril/valsartan	Sitagliptin	${\it Sacubitril/valsartan + sitagliptin}$
Glucose				
Baseline (T $= -60$ min) (mmol/L)	8.8 (7.8-9.9)	9.3 (7.6-10.9)	8.8 (7.8-9.8)	9.1 (7.6-10.6)
Peak (mmol/L)	11.7 (10.0-13.3)	13.4 (11.2-15.7)**	11.7 (10.1-13.3)	12.1 (10.0-14.3)
Insulin				
Baseline (T $= -60$ min) (pmol/L)	132 (70-194)	113 (87-139)	115 (79-152)	159 (95-224)
Peak (pmol/L)	444 (346-541)	424 (291-558)	440 (323-557)	443 (292-594)
C-peptide				
Baseline (T $= -60$ min) (pmol/L)	1323 (1016-1630)	1275 (1051-1499)	1297 (1041-1554)	1527 (1172-1883)
Peak (pmol/L)	2709 (2215 -3204)	3319 (2567-4070)*	2874 (2334-3415)	3199 (2542-4455)
Total GLP-1				
Baseline (T $= -60$ min) (pmol/L)	13 (10-15)	11 (10-12)	10 (8-12)	9 (6-10)
Peak (pmol/L)	21 (19-24)	24 (22-27)*	18 (15-21)	19 (17-22)
Intact GLP-1				
Baseline (T $= -60$ min) (pmol/L)	1 (0.08-2)	0.2 (0-1)	2.0 (1-3.0)*	1 (0.07-2)
Peak (pmol/L)	1 (1-2)	1 (0.1-1)	4 (2-5)*	9 (6-12)**
Glucagon				
Baseline (T $= -60$ min) (pmol/L)	11 (5-17)	9 (6-13)	9 (5-14)	9 (4-14)
Peak (pmol/L)	20 (15-25)	39 (28-50)***	15 (10-20)*	38 (24-53)
Total GIP				
Baseline (T $= -60$ min) (pmol/L)	12 (7-17)	8 (6-11)	9 (6-12)	8 (4-11)
Peak (pmol/L)	73 (62-83)	60 (53-66)	52 (44-60)*	41 (34-48)*
Intact GIP				
Baseline (T $= -60$ min) (pmol/L)	7 (3-11)	4 (2-6)	10 (7-14)	9 (5-14)
Peak	43 (34-51)	35 (28-42)	61 (52-70)*	45 (36-54)

Note: Data are means (95% CI). *p < .05, **p < .01 and ***p < .001 compared with the control day using a one-way ANOVA correcting for multiple testing by Sidak algorithm.

Abbreviations: GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide-1; T, Time.

Postprandial insulin responses did not differ significantly (p > .3) between study days (Figure 1C,D, Table 2, Figure S2), and nor did insulin-to-glucose-ratios (Figure S3).

3.3.2 | C-Peptide

Plasma concentrations of C-peptide at fasting were not significantly different (p > .2) between study days (Figure 1F, Table 2). Sacubitril/valsartan administered alone significantly increased postprandial C-peptide concentrations by 43% (iAUC) compared with the control day (p = .04) (Figure 1F, Table 2). Sacubitril/valsartan + sitagliptin numerically increased postprandial C-peptide compared with the control day (p = .10) (Figure 1E,F, Table 2). Sitagliptin and valsartan monotherapy had no significant effect on C-peptide concentrations (p > .1) (Figure 1E,F, Table 2, Figure S2).

The C-peptide to glucose ratio was not significantly different between groups (Figure S3).

To investigate further whether the observed increase of circulating C-peptide after sacubitril/valsartan could be explained by prevention of its cleavage by neprilysin, we performed an in vitro neprilysin degradation assay. In a time-course experiment, we mixed human recombinant neprilysin with a positive control (ANP) and C-peptide. Fifteen minutes after addition of recombinant neprilysin, ANP was partly degraded and was completely degraded after 60 min (Figure S4). In contrast, we observed no effect of recombinant neprilysin on C-peptide intensities, suggesting that C-peptide is not a neprilysin substrate.

3.4 | Glucagon-like peptide 1, glucagon and glucose-dependent insulinotropic polypeptide responses

3.4.1 | Total glucagon-like peptide-1

Plasma concentrations of total GLP-1 at fasting were not significantly different (p > .4) between study days (Figure 2A, Table 2).

Sacubitril/valsartan increased postprandial plasma concentrations of total GLP-1 by 22% (p < .05) compared with the control day (Figure 2B, Table 2). Sacubitril/valsartan + sitagliptin, sitagliptin alone or valsartan alone had no significant effect on postprandial concentrations of total GLP-1 (Figure 2A,B, Table 2, Figure S5).

FIGURE 1 (A,B) Glucose, (C,D) insulin and (E,F) C-peptide responses to a standardized mixed meal test, during control (black line and circle), sacubitril/valsartan treatment (red line and squire), sitagliptin treatment (blue line and upward triangle), sacubitril/ valsartan plus sitagliptin treatment (orange line and downward triangle). (A,C,E) Data are shown as mean ± SEM. (B,D,F) Calculated iAUC_{0-240 min}, control (black and white box), sacubitril/valsartan treatment (red box), sitagliptin treatment (blue box), sacubitril/valsartan plus sitagliptin treatment (orange box). Data are means ± Tukey whiskers with outliers shown as symbols. p < .05, ***p < .001 by iAUC₀₋₂₄₀ using a oneway ANOVA correcting for multiple testing by Sidak algorithm. N = 12(male subjects). iAUC, incremental area under the curve



3.4.2 | Intact glucagon-like peptide-1

Plasma concentrations of intact GLP-1 (7-36NH₂) were increased at fasting after sitagliptin compared with the control day and sacubitril/ valsartan (p = .03) (Figure 2C, Table 2). There was no significant difference in intact GLP-1 levels at baseline between other study days.

Sitagliptin significantly (p < .05) increased postprandial plasma concentrations of intact GLP-1 by 97% compared with the control day whereas sacubitril/valsartan alone did not (p = .9) (Figure 2C,D, Table 2). Sacubitril/valsartan combined with sitagliptin further enhanced plasma concentrations of intact GLP-1 by 370% resulting in a mean peak difference of 5 pmol/L (95% CI: 2-9) (p = .003) compared with sitagliptin (Figure 2C,D, Table 2). Adding sacubitril/valsartan to sitagliptin treatment had a synergistic effect on plasma concentrations of intact GLP-1 as evaluated by factorial analysis.

Valsartan alone had no effect on intact GLP-1 concentrations compared with the control day (p > .1) (Figure S5).

3.4.3 | Glucagon

Plasma concentrations of glucagon at baseline did not differ between study days (p > .9) (Figure 2E, Table 2).





FIGURE 2 Plasma concentrations of (A,B) total glucagon-like peptide-1 (GLP-1), (C,D) intact GLP-1 and (E,F) glucagon during a standardized mixed meal test. Control (black line and circle), sacubitril/valsartan treatment (red line and squire), sitagliptin treatment (blue line and upward triangle), sacubitril/valsartan plus sitagliptin treatment (orange line and downward triangle). (A,C,E) Data are shown as mean ± SEM. (B,D,F) Calculated iAUC_{0-240 min} control (black and white box), sacubitril/valsartan treatment (red box), sitagliptin treatment (blue box), sacubitril/ valsartan plus sitagliptin treatment (orange box). Data are means ± Tukey whiskers with outliers shown as symbols. *p < .05 by iAUC₀₋₂₄₀ using one-way ANOVA correcting for multiple testing by Sidak algorithm. N = 12 (male subjects). iAUC,

N = 12 (male subjects). IAUC, incremental area under the curve

Sacubitril/valsartan increased plasma glucagon by 351% (p = .002) and increased peak plasma glucagon concentration by 20 pmol/L (95% CI: 10.76-28.4; p = .0001) compared with the control day (Figure 2E,F, Table 2). Sitagliptin decreased peak plasma glucagon concentrations (p = .005) compared with the control day (Figure 2E,F, Table 2) but did not lower plasma concentrations of glucagon when co-administered with sacubitril/valsartan (p > .2).

Valsartan alone had no effect on postprandial glucagon concentrations compared with the control day (p = .9) (Figure 2E,D, Table 2, Figure S5).

The glucagon/insulin ratio increased postprandially after sacubitril/valsartan compared with the control day (p = .03) (Figure S6).

3.4.4 | Total glucose-dependent insulinotropic polypeptide

Fasting plasma concentrations of total GIP did not differ between the study days (Figure 3A, Table 2).

Treatment with sacubitril/valsartan combined with sitagliptin decreased postprandial plasma concentrations of total GIP by 43% (p = .01) compared with the control day, and by 21% compared with sitagliptin alone (Figure 3B, Table 2). Sacubitril/valsartan alone

FIGURE 3 Plasma concentrations of (A,B) total glucose-dependent insulinotropic polypeptide (GIP) and (C,D) intact GIP during a standardized mixed meal test. Control (black line and circle), sacubitril/valsartan treatment (red line and square), sitagliptin treatment (blue line and upward triangle), sacubitril/valsartan plus sitagliptin treatment (orange line and downward triangle). (A,C) Data are shown as mean ± SEM. (B,D) Calculated iAUC_{0-240 min}, control (black and white box), sacubitril/valsartan treatment (red box), sitagliptin treatment (blue box), sacubitril/ valsartan plus sitagliptin treatment (orange box). Data are means ± Tukev whiskers with outliers shown as symbols. **p < .01 by iAUC₀₋₂₄₀ using one-way ANOVA correcting for multiple testing by Sidak algorithm. N = 12 (male subjects), iAUC, incremental area under the curve



numerically lowered postprandial levels of total GIP compared with the control day (p = .2).

Valsartan alone had no effect on total GIP concentrations compared with the control day (p = .9) (Table 2, Figure S7).

3.4.5 | Intact glucose-dependent insulinotropic polypeptide

Fasting plasma concentration of intact GIP did not differ between study days (Figure 3C, Table 2).

Sacubitril/valsartan had no effect on plasma levels of intact GIP (p > .2) (Figure 3C, Table 2). Treatment with sacubitril/valsartan combined with sitagliptin did not affect plasma concentrations of intact GIP significantly compared with the control day but lowered intact GIP when compared with sitagliptin alone (Figure 3C,D, Table 2). Sitagliptin alone increased plasma concentrations of intact GIP by 112% and 75% when compared with the control day and with sacubitril/valsartan, respectively (both with p < .001) (Figure 3C,D, Table 2).

Valsartan alone had no effect on intact GIP (p > .9) (Figure S7).

3.5 | Heart rate and blood pressure, free fatty acids and triglycerides

Blood pressure, heart rate and serum free fatty acids and triglycerides did not differ significantly across the five study days (Figure S8). Heart rate increased on all five study days. On the two sacubitril/valsartan days the increment in heart rate persisted throughout the 240 min whereas heart rate returned to baseline with the remaining agents

3.6 | Neprilysin activity

Neprilysin activity was significantly reduced by more than 85% when sacubitril/valsartan was administered compared with the control day (p < .05) (Figure S9).

4 | DISCUSSION

Inhibitors of neprilysin have been reported to improve glucose tolerance in mice by GLP-1 receptor-dependent mechanism(s)¹⁴ and recently, in a post-hoc analysis of a randomized controlled trial, where treatment for 1 year with sacubitril/valsartan in patients with type 2 diabetes and heart failure was associated with reductions in HbA1c and a decreased need for insulin therapy.² The acute effect of combined neprilysin-inhibitor/angiotensin II receptor blocker treatment on glucose control has, to our knowledge, not been examined previously in patients with type 2 diabetes without heart failure. Our data suggest that acute neprilysin inhibition and angiotensin II receptor blocker treatment worsens rather than improves postprandial glucose control in patients with type 2 diabetes. In a subset of study participants, the angiotensin II receptor blocker valsartan was given alone with no effect on glucose tolerance or other metabolic markers suggesting that the acute metabolic effects observed during sacubitril/valsartan may be primarily related to the inhibition of neprilysin. Longer-term treatment with angiotensin II receptor blocker has been associated with improved insulin sensitivity¹⁵ but has in other short-term studies with obese individuals not been shown to affect insulin sensitivity or glucose tolerance acutely.¹⁶

Our findings suggest that long-term sacubitril/valsartan treatment in the patients with type 2 diabetes and heart failure may improve glycaemic control by mechanism(s) that differ from those elicited during acute administration; and our results suggest that they are independent of changes in entero-pancreatic hormone secretion (see Figure S10). The potentially beneficial chronic effects of sacubitril/valsartan on glucose homeostasis in patients with type 2 diabetes and heart failure are thus unclear, but may include increased physical activity because of improvements in heart function and or improvement in insulin sensitivity, as suggested in preclinical studies.^{3,14,17} The latter is, however, speculative and human data are needed to evaluate if long-term treatment with sacubitril/valsartan directly or indirectly affect insulin sensitivity in humans. Further studies evaluating the metabolic effects of angiotensin II receptor blocker in patients with type 2 diabetes also appear warranted.

Numerous bioactive peptides have been reported to be substrates for neprilysin cleavage, including the incretin hormone GLP-1.¹⁸ In this study, we found that a single dose of sacubitril/valsartan increased plasma concentrations of total GLP-1 (the sum of the metabolite GLP-1 9-36NH₂ and the active hormone GLP-1 7-36NH₂). These data are in line with previous in vitro data and a study in healthy individuals showing that inhibitors of neprilysin improve the stability of circulating endogenous GLP-1.⁷ The mode of action of neprilysin includes a mid-sequence cleavage point, in contrast to the cleavage by DPP-4 from the N-terminus. Esser et al. recently reported that neprilysin inhibition improves glucose control in a mouse model of insulin deficiency in a GLP-1 receptor-dependent manner.¹⁴ However, the effect of neprilysin on glucose tolerance in that study appeared dependent on the route of administration, as neprilysin inhibition only improved glucose control when glucose was administered intravenously, but not orally, which is surprising, but suggests that the effect was gut-independent.¹⁴ In contrast, our data indicate that acute inhibition of neprilysin in humans results in hyperglycaemia in patients with type 2 diabetes, independent of its effects on plasma concentrations of GLP-1. The DPP-4 inhibitor sitagliptin had no significant acute effect on glucose tolerance in this study but did seem to counteract the glucose deterioration induced after a single dose of sacubitril/valsartan. Although, here, we did not find any effect of sitagliptin on insulin levels alone or in combination with sacubitril/valsartan. The lack of effect of sitagliptin alone may potentially be explained by the glucose-dependent action of GLP-1 as study participants had well controlled type 2 diabetes reflected by HbA1c levels and that the majority were treated with metformin only.

Notably, we found that a single dose of sacubitril/valsartan caused a more than three-fold elevation of the plasma concentrations of glucagon, resulting in overt hyperglucagonaemia. We have previously showed that neprilysin cleaves glucagon both in vivo in healthy individuals, and in in vitro experiments.^{13,19} While considering the powerful effects of glucagon on hepatic glycogenolysis and gluconeogenesis, it seems probable that the hyperglucagonaemia found after neprilysin inhibition may explain the impairment in glucose tolerance of patients with type 2 diabetes in our study. The effect on glucose concentrations by sacubitril/valsartan could in theory also be

explained by increased activity of the sympathetic nervous system. In line with this, heart rate increased during all experimental days and returned to baseline; however, when sacubitril/valsartan was administered the increase in heart rate tended to persist (~5 beats/min) potentially reflecting increased activity of the sympathetic nervous system. However, these changes were not statistically significant between groups. Our study is limited by the fact we did not measure noradrenalin or other activity markers of the sympathetic nervous system. We cannot exclude that an increased activity of the sympathetic nervous system may have contributed to the effects on glucose tolerance but find it unlikely.

In this study, we also investigated the effect of sacubitril/ valsartan on the other incretin hormone, GIP. Consistent with our findings in healthy individuals,⁷ plasma concentrations of total GIP were significantly lowered after a single dose of sacubitril/valsartan but not after valsartan alone. The underlying mechanism(s) for this is unknown, but given the physiological importance of GIP on glucose control the reduced levels of GIP may contribute to the impaired glucose tolerance after sacubitril/valsartan, although the insulinotropic actions of GIP seem to be impaired in patients with type 2 diabetes.²⁰

We found increased C-peptide levels that could not be explained by the direct effects of neprilysin inhibition per se (cleavage) but may either reflect increased β -cell secretion or altered renal elimination. Current studies with sacubitril/valsartan do, however, not show negative effect on renal function.²¹ We did not observe changes in the Cpeptide/glucose ratio, suggesting that the increased glucose itself may account for the altered C-peptide levels. One would, however, expect that plasma concentrations of insulin would increase simultaneously with C-peptide, but this was not the case. It cannot be excluded that other proteases are required for a neprilysin-induced cleavage of C-peptide and this warrants further investigation.

5 | CONCLUSIONS

Based on a previous study in healthy individuals, we hypothesized that acute administration of sacubitril/valsartan combined with a DPP-4 inhibitor would lower postprandial glucose concentrations in patients with type 2 diabetes by enhancing active GLP-1 levels. Although the latter was indeed confirmed, glucose tolerance worsened in our patients with type 2 diabetes possibly because of a concomitant increase in plasma glucagon levels, which probably increased hepatic glucose production. Because of the absence of an acute glucose-lowering effect in the present study, long-term studies are warranted to explore the mechanism(s) behind the previously observed glucose-lowering effects of sacubitril/valsartan in patients with type 2 diabetes and heart failure.

AUTHOR CONTRIBUTIONS

NJWA, PP, JPG, FG, CFD, JJH, SM and KNB-M formulated the hypothesis, designed the study, wrote the protocol and obtained ethical and data approvals. AM, CM, LLG, EBR, SM and KNB-M recruited study participants. AM and CM performed screening and the executed the five experimental study days. SASK and LHH performed the in vitro study and mass spectrometry. NJWA performed biochemical analyses. NJWA and AM performed statistical analyses and drafted figures. NJWA, AM and KNB-M wrote the manuscript. All authors read, revised and accepted the submitted manuscript. NJWA is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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CONFLICT OF INTEREST

NJWA has received speaker fees from MSD as subsidiary of MERCK and Mercodia, research support from Novo Nordisk A/S and Mercodia. CFD has received consultancy/lecture fees from companies with an interest in developing and marketing incretin-based therapies for treatment of type 2 diabetes (Boehringer Ingelheim, Lilly, Merck/ MSD, Novo Nordisk). Spouse holds stock in Merck/MSD. JJH and LLG are members of advisory boards for Novo Nordisk A/S and has received lecture fees from the same company. Other authors declare that they have no competing interests.

PEER REVIEW

The peer review history for this article is available at https://publons. com/publon/10.1111/dom.14789.

DATA AVAILABILITY STATEMENT

Data available on request from the authors

PRIOR PRESENTATION

The study was presented at the European Association for the Study of Diabetes (EASD) Conference 2020 (ePoster no. 625).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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