DPP-4 inhibition has no acute effect on BNP and its N-terminal pro-hormone measured by commercial immune-assays. A randomized cross-over trial in patients with type 2 diabetes

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Abstract

Background: Use of dipeptidyl peptidase-4 inhibitors (DPP-4-i) for the treatment of type 2 diabetes (T2D) has been associated with a possible increase in the risk for heart failure (HF). B-type natriuretic peptide (BNP), which is both a biomarker of HF and a hemodynamically active hormone, is a substrate of DPP-4. We herein tested the acute effects of the DPP-4i linagliptin on BNP and NT-proBNP in a cross-over placebo-controlled trial in patients with T2D with and without chronic kidney disease (CKD).

Methods: B-type natriuretic peptide and NT-proBNP were measured using commercially available clinical-grade immune-assays at baseline and at the end of a 4-day treatment with placebo and linagliptin. Changes from baseline during each treatment arm, as well as placebo-subtracted effects of linagliptin on BNP and NT-proBNP were calculated.

Results: 46 patients completed the study, 18 of whom were affected by CKD. Baseline BNP and NT-proBNP levels increased with age, were elevated in CKD patients, and inversely correlated with estimated glomerular filtration rate. No significant change was detected in BNP and NT-proBNP levels after treatment with linagliptin or placebo in patients with or without CKD. Only in CKD patients the placebo-subtracted effect of linagliptin indicated a significant reduction in NT-proBNP levels, but this finding was not statistically robust.

Conclusions: Acute treatment with a DPP-4i exerts no clinically-meaningful effects on BNP and NT-proBNP. As routinely used immunoassays do not discriminate between intact/active and cleaved BNP, these data cannot rule out an effect of DPP-4i on HF pathophysiology.

Trial registration NCT01617824

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identified as endogenous physiological substrates in vivo [3].

Concerns have been raised on the possibility that DPP-4i increase the risk of heart failure (HF), but the mechanisms are largely unknown [4, 5]. Intact B-type natriuretic peptide (BNP1–32) is cleaved by DPP-4, generating BNP3–32 [6]. BNP, which is produced by cardiomyocytes in response to hemodynamic stress and neuro-hormonal stimulation, is a clinical-grade biomarker of HF [7]. In turn, BNP is involved in the pathophysiology of HF, as it induces vasodilation and natriuresis, thereby antagonizing the effects of angiotensin-II [8, 9]. BNP concentrations are reduced in people with obesity, insulin resistance, and diabetes, and this deficiency may contribute to their cardiovascular risk [10]. Thus DPP-4i may exert beneficial effects on cardiac function, by increasing the proportion of intact/active BNP1–32 versus cleaved BNP3–32, in addition to GLP-1 mediated cardioprotection [11].

BNP1–32 derives from proBNP (108 amino-acid) after removal of an N-terminal (NT) fragment of 76 amino-acids by pro-hormone convertases [12]. Although BNP and NT-proBNP are released at equimolar concentrations, the half-life of the NT-proBNP in the circulation is longer, resulting in higher concentrations. In patients with diabetes, despite a possible reduction of BNP, the clinical predictive capacity of NT-proBNP has been shown to be preserved [13, 14]. Interestingly, also proBNP1–108 and NT-proBNP1–76 are candidate substrates of the enzymatic activity of DPP-4, as they have proline in the second N-terminal position, where the exopeptidase activity of DPP-4 locates. Commercially available immuno-assays are presumably unable to distinguish between BNP1–32 and BNP3–32 [15], and they may even detect proBNP1–108/3–108, but not NT-proBNP [16]. As compared to BNP1–32, truncated BNP3–32 appears to have equal cGMP activating properties in vitro [17], but lower activity in vivo [18], probably because of a higher susceptibility to degradation by other peptidases. Specificity of NT-proBNP immunoassays is unknown, but epitopes recognized by monoclonal antibodies do not appear to span the first 2 N-terminal residues. Based on these considerations, the net effect of a DPP-4i therapy on diagnostic BNP and NT-proBNP determinations is unpredictable (Fig. 1).

Available data on the effects of DPP-4i on proBNP-derived peptides in T2D mostly come from large clinical trials wherein NT-proBNP levels were measured years after therapy with a DPP-4i or placebo [19, 20]. Rather, if DPP-4i has direct effects on proBNP processing, this should be detectable within a short time frame. To address the acute effects of DPP-4i on BNP and NT-proBNP, we used samples from a placebo-controlled cross-over trial testing the effects of a 4-day therapy with the DPP-4i inhibitor linagliptin on humoral factors [21]. Differently from other DPP-4i, linagliptin has no renal excretion, and is thereby particularly suitable for the treatment of patients with chronic kidney disease (CKD) [22, 23], which is a major risk factor for HF [24].

**Methods**

**Study design**

The NCT01617824 was a randomized, single-blind, placebo-controlled, cross-over study designed to test the acute effects of the DPP-4 inhibitor linagliptin on cytokines, hormones, and inflammatory mediators. The

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![Diagram](https://via.placeholder.com/150)

**Fig. 1** Sequential cleavage of proBNP to originate BNP, NT-proBNP and their by-products. Biological activity on cardiac function is reported. DPP-4 can cleave the 2 N-terminal residues of proBNP, BNP, and NT-proBNP, generating inactive or less active peptides.
primary study results have been published before [21]. Briefly, T2D patients with or without CKD, received a 4-day treatment with linagliptin 5 mg and placebo in a random order with a 14-day wash-out period. Before and at the 5th day of each treatment period, fasting blood samples were drawn. Aliquots of EDTA and heparin plasma were separated and stored at −80 °C until analyses. CKD was defined as an estimated glomerular filtration rate (eGFR) of less than 60 ml/min/1.73 m², based on the CKD-EPI formula [25] and graded according to the Kidney Disease Outcomes Quality Initiative (KDOQI) [26]. With this design, we were able to detect significant changes in DPP-4 activity, levels of intact (uncleaved) GLP-1 and SDF-1α, along with an increase in CD34⁺KDR⁺ cells, reflecting a biological consequence of elevated SDF-1α [21], an effect that was observed also for saxagliptin [27]. For this study, untouched frozen aliquots of plasma were thawed and used for the quantification of BNP and NT-proBNP.

Analytical methods
B-type natriuretic peptide was quantified in EDTA plasma using a chemiluminescent, microparticle-capture immunoassay (Abbott Diagnostics kit, #8K28) on the modular automated ARCHITECT iSystem platform. The range of plasma BNP concentrations revealed using this assay is 10–5000 pg/ml. The coefficient of variation (CV), as reported by the manufacturer, is <5%. This BNP assay results in no cross-reactivity with ANP, Angiotensin-I, Angiotensin-II, Angiotensin-III, CNP, and NT-proBNP.

NT-proBNP was quantified in heparinised plasma using a solid-phase two-site chemiluminescent immunoassay (Siemens IMMULITE 1000 Turbo). The sensitivity of this assay is 15 pg/ml, with a reportable range up to 35,000 pg/ml. The CV reported by the manufacturer is 9%. No cross-reactivity has been detected with ANP, NT-proANP, BNP, CNP, Adrenomedullin, Angiotensin-I, Angiotensin-II, Angiotensin-III, CNP, and Arg-Vasopressin.

Statistical analysis
Data are expressed as mean ± standard error if normal or as median (interquartile range) if not normal. Normality was checked using the Shapiro–Wilks test and non-normal variables were log-transformed before analysis. Within-group changes in continuous variables were analyzed using the paired two-tail Student’s t test. For each patients in each group of treatment order, we calculated the effect of placebo, the effect of linagliptin, and the placebo-subtracted effect of linagliptin. The generalized linear model (GLM) was used to analyze the effect of treatment and order by the cross-over design. Statistical significance was accepted at p < 0.05 and SPSS version 22.0 was used.

Sample size was originally chosen to achieve a 80% power to detect a significant difference in the primary end-point (a difference in circulating CD34⁺KDR⁺ cells), which was fully satisfied. Based on within-patients standard deviations of 37% for BNP (26 pg/ml) and 40% for NT-proBNP (227 pg/ml), we calculated a priori that this study had 80% power to detect a treatment difference at a two-sided 0.05 significance level, if the true difference between treatments was 22% for BNP (15 pg/ml) and 24% for NT-proBNP (136 pg/ml).

Results
Characteristics of study patients
A total of 46 patients completed the study. Detailed baseline clinical characteristics of the participants have been reported previously [21] and are herein summarized in Table 1. There was no difference between patients randomized to the placebo-linagliptin (n = 22) or the linagliptin-placebo (n = 24) treatment order. No mild or severe adverse event was reported during treatment or wash-out and no change in fasting metabolic variables (glucose, triglycerides and fatty acids) was observed [21].

Baseline values of BNP and NT-proBNP
As the distributions of BNP and NT-proBNP were highly skewed, data are presented as median (IQR) and values were log-transformed before statistical testing. The median baseline plasma BNP level was 20.4 pg/ml (IQR 10.0–43.3). BNP was below threshold (10 pg/ml) in n = 15 patients (32.6%) and was above the decisional cut-off (100 pg/ml) [28] in 6 patients (13.0%). BNP levels increased with age (r = 0.40; p = 0.003), were higher in patients with CKD than in those without (43.1 [IQR 22.1–98.5] versus 12.5 [IQR 10.0–23.0] pg/ml; p = 0.0022) and were inversely correlated with eGFR (r = −0.45; p < 0.001).

The median baseline NT-proBNP level was 101.0 pg/ml (IQR 35.3–314.8) and n = 5 patients (10.9%) had values above the decisional cut-off (900 pg/ml) [28]. NT-proBNP levels increased with age (r = 0.52; p < 0.001), were higher in patients with CKD than in those without (238.5 [IQR 115.0–554.8] versus 44.0 [IQR 24.3–101.0] pg/ml; p < 0.001) and were inversely correlated with eGFR (r = −0.50; p < 0.001).

Levels of BNP and NT-proBNP were highly correlated (r = 0.94).

Effects of DPP-4 inhibition on BNP and NT-proBNP
Overall, no significant change versus baseline was observed in BNP and NT-proBNP levels after treatment with linagliptin or placebo (Fig. 2). The
No carry-over effect was noted for both BNP and NT-proBNP. No correlation was detected between BNP or NT-proBNP and DPP-4 activity, nor between change in BNP or NT-proBNP and change in DPP-4 activity.

**Discussion**

We show that therapy with a DPP-4i has no acute effects on BNP and NT-proBNP levels measured with routine diagnostic immuno-assays. This study was not designed to test the acute effects of DPP-4i on cardiac function, but our findings re-assure on the safety of DPP-4i concerning diagnosis and prognostic evaluation of HF.

The clinical relevance of the interplay between DPP-4i and BNP/NT-proBNP levels has emerged after publication of the results of SAVOR-TIMI trial, wherein patients treated with the DPP-4i saxagliptin exhibited a significant 27% excess risk of hospitalization for HF compared to placebo [19, 29]. Meta-analyses of randomized controlled trials were unable to rule out the concern that DPP-4i therapy may favour HF [4, 5]. Real-world data did not confirm an association between DPP-4i and hospitalization for HF [30, 31], nor show adverse prognosis in HF patients treated with DPP-4i [32, 33]. Furthermore, the eventual mechanisms remain elusive. In the SAVOR-TIMI trial, the risk of HF associated with saxagliptin therapy was almost exclusively observed in patients with a baseline NT-proBNP level within the most elevated quartile [19]. During a follow-up of about 2 years, NT-proBNP levels increased in both the placebo and saxagliptin group, but the increase was slightly blunted by saxagliptin [19]. The relevance of this finding is limited because exceeding HF cases in saxagliptin-treated patients were observed only in the first 6 months of therapy [19]. In another placebo-controlled trial conducted on T2D patients after an acute coronary event, the DPP-4i alagliptin was associated with a non-significant increase in the risk of hospitalization for HF [34]. During an average 1.5 year follow-up, NT-proBNP concentrations decreased significantly and similarly in the two groups [20]. Our study shows for the first time that DPP-4i therapy may favour HF [4, 5]. Real-world data did not confirm an association between DPP-4i and hospitalization for HF [30, 31], nor show adverse prognosis in HF patients treated with DPP-4i [32, 33].

Chronic kidney disease is one of the strongest risk factors for HF [24]. As BNP and NT-proBNP levels were higher in CKD patients, the amplitude of their excursions after DPP-4i or placebo was also larger. Although consistent with a study showing that linagliptin decreased BNP in an experimental model of uremic cardiomyopathy [35], the modest placebo-subtracted effect of DPP-4i on NT-proBNP reduction we observe in CKD patients

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**Table 1 Baseline characteristics of study patients**

| Variable                  | All patients |
|---------------------------|--------------|
| Number                    | 46           |
| Age, years                | 63.7 ± 1.3   |
| Sex male, %               | 71.7         |
| Body mass index, kg/m²    | 31.1 ± 0.7   |
| Waist, cm                 | 105.4 ± 2.2  |
| HbA1c, %                  | 7.6 ± 0.2    |
| (mmol/mol)                | (60 ± 2)     |
| Risk factors              |              |
| Smoking habit, %          | 13.0         |
| Hypertension, %           | 89.1         |
| Total cholesterol, mg/dl  | 165.3 ± 5.5  |
| HDL cholesterol, mg/dl    | 49.9 ± 2.2   |
| LDL cholesterol, mg/dl    | 91.6 ± 5.1   |
| Triglycerides, mg/dl      | 1192 ± 8.5   |
| Albumin/creatinine ratio (mg/g) | 1295 ± 44.1 |
| Creatinine, mg/dl         | 1.11 ± 0.06  |
| eGFR, ml/min/1.73 mq      | 75.5 ± 3.9   |

Complications

| Retinopathy, %            | 28.2         |
| Neuropathy, %             | 17.9         |
| Coronary artery disease, %| 30.4         |
| Peripheral arterial disease, % | 21.7       |
| Cerebrovascular disease, % | 47.8         |

Medications

| Metformin, %              | 65.2         |
| Sulphonylurea, %          | 6.5          |
| Repaglinide, %            | 4.3          |
| Pioglitzone, %            | 6.5          |
| Insulin, %                | 43.4         |
| ACE inhibitors/ARBs, %    | 76.1         |
| Other anti-hypertensives, %| 78.2         |
| Statin, %                 | 80.4         |
| Anti-platelet agents, %   | 56.5         |

Data are presented as mean ± standard error, or as percentage, where appropriate. More details can be found in [21].
Fig. 2  BNP and NT-proBNP levels during treatment with placebo and linagliptin. Data are presented as baseline (pre) and end-of-treatment (post) values (a, d), and change from baseline (b, e) during placebo or linagliptin. c f Show changes from baseline in BNP and NT-proBNP, respectively, in patients with (n = 18) CKD and in those without (n = 28; Ctrl). *p<0.05. The box plot shows median and IQR, whereas whiskers indicate Tukey range.

Table 2  BNP and NT-proBNP levels, expressed as median (IQR) during treatment with placebo or linagliptin

|               | Placebo        | Linagliptin   | Placebo-subtracted change |
|---------------|----------------|---------------|----------------------------|
|               | Pre            | Post          | Change                     | Pre            | Post          | Change                     |
| BNP           |                |               |                            |                |               |                            |
| All           | 22.9 (10.0–42.8) | 21.3 (10.0–52.6) | 0.0 (–0.9 to 15.2)        | 19.6 (10.0–44.3) | 22.6 (10.0–39.3) | 0.0 (–2.7 to 7.8)          |
| No CKD        | 11.4 (10.0–23.3) | 11.9 (10.0–29.5) | 0.0 (–0.9 to 0.3)         | 10.6 (10.0–21.8) | 12.5 (10.0–27.4) | 0.0 (0.0–4.6)              |
| CKD           | 38.5 (26.8–80.3) | 56.3 (24.6–107.1) | 7.3 (–0.7 to 26.0)        | 44.2 (20.3–129.0) | 37.4 (25.0–78.9) | 0.0 (–9.5 to 12.1)         | 0.0 (–19.0 to 1.7)         |
| NT-proBNP     |                |               |                            |                |               |                            |
| All           | 101.0 (36.5–314.5) | 122.0 (38.0–267.0) | 2.0 (–32.0 to 30.5)       | 101.0 (44.0–299.0) | 78.0 (36.0–272.0) | –3.5 (–27.5 to 19.3)       |
| No CKD        | 46.0 (26.0–101.0) | 57.0 (20.0–167.0) | 0.0 (–12.0 to 20.0)       | 51.0 (28.5–121.5) | 41.0 (26.5–99.0) | 3.0 (–17.5 to 17.3)        |
| CKD           | 218.5 (108.8–554.8) | 261.0 (130.0–696.5) | 4.5 (–57.8 to 109.3)      | 238.5 (107.5–611.5) | 184.0 (109.0–483.0) | –17.0* (–59.3 to 23.0)     |

* Significantly different from placebo treatment (p < 0.05 at paired t test on log-transformed data or Mann–Whitney test)

* Significantly different from zero
had very low statistical power, was no longer significant after adjusting for multiple testing, and is unlikely to be of any clinical meaning, as NT-proBNP is not biologically active.

The present study has limitations inherent to the small sample size, the fact that a minority of patients had CKD, thereby lowering power in this subgroup, and the lack of data in patients with decompensated HF. Finally, more subtle changes in BNP and NT-proBNP induced by DPP-4i may have been missed, since the study was powered for a minimal detectable change of 22 and 24%, respectively. Since clinical-grade commercially available immuno-assays do not distinguish the intact and cleaved forms of BNP and NT-proBNP, our data provide no clear indication of whether DPP-4i interferes with the in vivo processing of the two peptides, and whether it intervenes in the pathophysiology of HF. However, any eventual significant change in the relative proportion of BNP$_{1-32}$ and BNP$_{3-32}$ or in the proportion of NT-proBNP$_{1-76}$ and NT-proBNP$_{3-76}$ induced by DPP-4i may nonetheless result in modifications of immune-reactive (total) BNP and NT-proBNP levels, respectively. This has been shown for GLP-1 and SDF-1α [21], possibly reflecting compensatory secretion and/or changes in sequential cleavage by different peptidases. Furthermore, experimental studies suggest that linagliptin exerts favourable effects on ischemia–reperfusion injury [36], which in the long-term can translate into protection from HF.

**Conclusion**

Although exact discrimination of the various proBNP-derived peptides will require sophisticated, time-consuming and costly mass spectrometric approaches [15], data obtained with diagnostic assays indicate that DPP-4i has no clinically appreciable effects on BNP and NT-proBNP. Further studies will be needed to dissect whether DPP-4i interferes with the biological action of BNP and whether this is linked to HF risk in patients with T2D.

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None.

**Competing interests**

GF and AA report receiving lecture fees and honoraria from manufacturer of Linagliptin and other DPP-4 inhibitors. The other authors report no competing interests.

**Availability of data and materials**

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

**Ethics approval and consent to participate**

The study was approved by the Ethical Committee of the University Hospital of Padova (prot. number). All participants provided written informed consent.

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