

# Treatment with metformin and a dipeptidyl peptidase-4 inhibitor elevates apelin levels in patients with type 2 diabetes mellitus

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**Background:** The objective of this study was to assess the effects of metformin monotherapy or combined treatment with a dipeptidyl peptidase-4 inhibitor (vildagliptin) on apelin levels in patients with type 2 diabetes mellitus.

**Methods:** Twenty-five patients with poor glycemic control (glycosylated hemoglobin >6.5% [48 mmol/mol]) taking 1,000 mg of metformin daily and 25 healthy controls matched for age and body mass index were enrolled in this study. Anthropometric parameters, glycemic and lipid profile, insulin resistance (homeostasis model assessment of insulin resistance index), and apelin levels were measured at baseline and at 12-week and 24-week visits.

**Results:** At baseline, apelin levels were higher in the T2DM patients than in the controls (1.93±1.81 ng/mL versus 6.09±4.90 ng/mL;  $P<0.05$ ). After 12 weeks, when vildagliptin was added, fasting blood glucose and glycosylated hemoglobin decreased, and apelin levels increased further (from 6.09±4.90 ng/mL to 24.23±12.59 ng/mL;  $P<0.05$ ). Follow-up at 24 weeks showed no further improvement in the glycemic profile and no further increase in apelin levels.

**Conclusion:** Both metformin and vildagliptin favorably changed glycemic indices and apelin levels. For patients inadequately controlled on a low dose of metformin, addition of vildagliptin may be helpful.

**Keywords:** glucagon-like peptides, glucose-dependent insulinotropic polypeptide, antidiabetic drug, adipocytokine

## Introduction

Apelin is a novel adipocytokine produced by white adipose tissue and binds the APJ receptor with high affinity.<sup>1</sup> Apelin and the APJ receptor are expressed in pancreatic islet cells,<sup>2</sup> and appear to be involved in energy metabolism. Many studies show that apelin plays a role in the regulation of glucose homeostasis, glucose-stimulated insulin secretion, and even insulin sensitivity.<sup>3-7</sup> These observations suggest that apelin has an important role in diabetes mellitus, not only as a therapeutic target,<sup>8</sup> but also as a biomarker.

Studies have reported decreased circulating apelin levels in patients with newly diagnosed and untreated type 2 diabetes mellitus (T2DM).<sup>9</sup> Our research group has also reported low plasma apelin levels in Chinese people with newly diagnosed T2DM.<sup>10</sup> Although experimental research supports the effects of this adipocytokine on insulin resistance, clinical studies evaluating the differential effects of antidiabetic drugs on plasma levels of apelin are lacking. Both metformin and dipeptidyl peptidase (DPP)-4 inhibitors improve glycemic control<sup>11</sup> and act on adipose tissue by improving the dysregulated “adipocytokine” profile in the insulin-resistant state.<sup>12,13</sup> Therefore, studies on the effects of pharmaceutical interventions, especially metformin and DPP-4 inhibitors, on apelin levels are warranted.

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## Materials and methods

### Subjects and study design

Twenty-five patients with T2DM and 25 healthy controls matched for age and body mass index (BMI) were enrolled in this study. Study inclusion criteria included already being treated with metformin (1,000 mg/day, Sino-American Shanghai Squibb Pharmaceuticals Ltd, Shanghai, People's Republic of China) alone for at least 4 weeks but without adequate glycemic control, ie, glycosylated hemoglobin ( $HbA_{1c}$ )  $>6.5\%$  (48 mmol/mol). None of the diabetic patients had acute or chronic complications. A DPP-4 inhibitor (vildagliptin 100 mg/day, Novartis Europharm Ltd, Horsham, UK) was added for at least 24 weeks. No patient had clinical evidence of cardiovascular disease (coronary, peripheral, carotid artery) or any other major chronic disease (autoimmune, life-threatening). None of female patients had polycystic ovary syndrome. All participants were instructed not to change their dietary habits or daily activities during the study period. At the 12-week visit, the patients were excluded if their  $HbA_{1c}$  was  $>9\%$  and if they had received rescue therapy. All procedures were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Declaration of Helsinki. This study was approved by the ethics committee of Minhang Hospital affiliated to Fudan University. Informed consent was obtained from all patients before inclusion in the study.

### Blood analyses

Blood samples were collected between 8 am and 8.30 am after 12 hours of fasting. The tubes were promptly centrifuged, and the plasma was separated and stored at  $-80^{\circ}\text{C}$ . All samples

were run in the same assay. Plasma glucose, urea, creatinine, uric acid, total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglycerides, alkaline phosphatase, aspartate aminotransferase, and alanine aminotransferase were measured on an automatic enzymatic analyzer.  $HbA_{1c}$  was determined by standard procedures. Insulin was measured by radioimmunoassay. The homeostasis model of assessment insulin resistance index was calculated. Plasma levels of apelin (human apelin-36) were assayed using a commercially available enzyme immunoassay kit (Phoenix Pharmaceuticals Inc, Burlingame, CA, USA) according to the manufacturer's instructions.

### Statistical analysis

The results are presented as the mean  $\pm$  standard deviation or as a ratio. The Kolmogorov–Smirnov test was used to determine the distribution characteristics of variables and Levene's test was used to evaluate the equality of variance. Comparisons between and within groups were performed by independent-samples *t*-test and paired-samples *t*-test, respectively. All tests were two-tailed. The relationship between variables was analyzed by Spearman's rho correlation. Differences and correlations were considered to be statistically significant at  $P < 0.05$ . Statistical Package for the Social Sciences version 17.0 software (SPSS Inc, Chicago, IL, USA) was used for the statistical analysis.

### Results

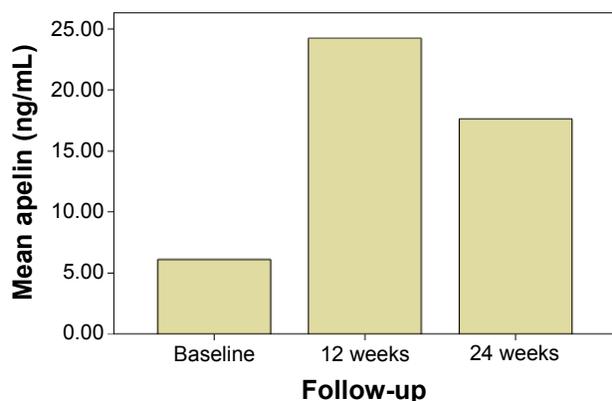
In our study, 25 patients with T2DM treated by metformin (1,000 mg/day) alone were initially recruited. One patient was lost to follow-up and excluded from the statistical analysis. Patient characteristics and laboratory data for the study

**Table 1** Characteristics and laboratory data for study participants

	Metformin group n=25	Control group n=25	P-value
Sex (male/female)	17/8	11/14	0.087
Age (years)	52.52 $\pm$ 9.16	52.84 $\pm$ 9.34	0.903
BMI (kg/m <sup>2</sup> )	24.93 $\pm$ 3.50	25.06 $\pm$ 2.60	0.887
Waist (cm)	91.75 $\pm$ 10.81	86.98 $\pm$ 5.44	0.081
WHR	0.93 $\pm$ 0.07	0.90 $\pm$ 0.04	0.125
Systolic BP (mmHg)	122.96 $\pm$ 17.46	119.20 $\pm$ 9.82	0.355
Diastolic BP (mmHg)	75.42 $\pm$ 13.75	80.68 $\pm$ 5.57	0.083
$HbA_{1c}$ (%)	7.43 $\pm$ 0.61	5.73 $\pm$ 0.24	0.000*
TC (mmol/L)	4.55 $\pm$ 0.78	4.14 $\pm$ 0.61	0.048*
TG (mmol/L)	1.78 $\pm$ 0.79	0.99 $\pm$ 0.40	0.000*
HDL-C (mmol/L)	1.16 $\pm$ 0.24	1.40 $\pm$ 0.30	0.004*
LDL-C (mmol/L)	2.79 $\pm$ 0.58	2.35 $\pm$ 0.50	0.007*
Apelin (ng/mL)	6.09 $\pm$ 4.90	1.93 $\pm$ 1.81	0.000*

**Notes:** Data presented as mean  $\pm$  standard deviation or as a ratio. \*P-value  $<0.05$ .

**Abbreviations:** BP, blood pressure; BMI, body mass index; WHR, waist to hip ratio;  $HbA_{1c}$ , glycosylated hemoglobin; TG, triglycerides; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol.



**Figure 1** Comparison of apelin levels after vildagliptin treatment.

participants are shown in Table 1. The two groups did not differ in age, sex, BMI, waist, or blood pressure.

The plasma apelin level was significantly higher in the metformin group than in controls ( $1.93 \pm 1.81$  ng/mL versus  $6.09 \pm 4.9$  ng/mL;  $P < 0.05$ ). After 12 weeks of add-on vildagliptin therapy, fasting blood glucose levels and HbA<sub>1c</sub> were lower than at baseline. Apelin increased significantly by 18.15 ng/mL from baseline ( $6.09 \pm 4.90$  ng/mL versus  $24.23 \pm 12.59$  ng/mL;  $P = 0.029$ ). Patients were followed up to 24 weeks, by which time the apelin levels decreased, albeit not significantly ( $24.23 \pm 12.59$  ng/mL versus  $17.62 \pm 8.51$  ng/mL;  $P = 0.975$ ). The apelin levels are shown in Figure 1. Other clinical and biochemical characteristics are shown in Table 2. No severe hypoglycemia events were observed. Other noteworthy adverse effects, such as cardiovascular events

or heart failure, were not reported during the study period. We also searched for correlations between apelin and the other parameters, but found no correlation between blood apelin levels and BMI, lipid levels, or insulin resistance (Table 3).

## Discussion

Apelin signaling may have an important role in the pathophysiology of several diseases, including hypertension, heart failure, cardiovascular disease, T2DM, and obesity, although its effects and functions are still unclear. However, increasing evidence suggests that apelin is involved in regulation of multiple physiological functions, including food intake, blood pressure, and glucose utilization.<sup>14,15</sup>

Our study demonstrated a remarkable increase in apelin levels in response to treatment with metformin in T2DM when compared with healthy controls. A plausible explanation is that metformin triggers secretion of apelin via adenosine 5' monophosphate-activated protein kinase, leading to alleviation of insulin resistance.<sup>16</sup>

This study demonstrates the positive effects of vildagliptin on plasma apelin levels in patients with T2DM, and is the first study to our knowledge to show a marked increase in apelin concentrations by add-on vildagliptin treatment. The effect was sustained until 24 weeks when compared with apelin levels at baseline. As we know, DPP-4 inhibitors inhibit DPP-4-dependent inactivation of glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1

**Table 2** Clinical and biochemical parameters at baseline and at the end of the study

	Baseline	12 weeks	24 weeks	P1 n=25	P2 n=24	P3 n=24
HbA <sub>1c</sub> (%)	7.43±0.61	6.78±0.74	6.82±0.73	0.005*	0.008*	0.057
FPG (mmol/L)	7.35±0.85	6.53±0.76	6.58±1.13	0.037*	0.041*	0.12
FINS (μU/mL)	7.74±4.38	8.12±4.12	9.04±4.08	0.545	0.890	0.890
HOMA-% B	48.66±21.10	56.43±26.32	64.88±34.44	0.656	0.481	0.481
HOMA-IR	1.29±0.72	1.38±0.59	1.47±0.66	0.872	0.673	0.958
TC (mmol/L)	4.55±0.78	4.78±0.82	4.47±0.48	0.812	0.465	0.873
TG (mmol/L)	1.78±0.79	2.03±0.67	1.98±0.78	0.954	0.115	0.665
HDL-C (mmol/L)	1.16±0.24	1.17±0.28	1.18±0.29	0.253	0.381	0.918
LDL-C (mmol/L)	2.79±0.58	2.95±0.72	2.72±0.41	0.330	0.901	0.845
Cr (μmol/L)	56.74±11.20	57.58±15.46	56.44±9.75	0.232	0.203	0.270
UA (μmol/L)	321.83±8.56	312.00±116.01	305.11±80.03	0.375	0.547	0.123
ALT (U/L)	21.30±4.06	22.08±4.40	22.33±4.61	0.950	0.069	0.869
AST (U/L)	20.09±5.48	21.92±5.42	21.33±4.97	0.403	0.064	0.509
ALP (U/L)	75.17±23.54	72.83±27.30	77.22±22.93	0.825	0.634	0.236
r-GT (U/L)	31.74±15.72	31.00±18.99	30.33±16.29	1.00	0.662	0.478

**Notes:** Data presented as mean ± standard deviation or as a ratio. \* $P$ -value  $< 0.05$ .

**Abbreviations:** FPG, fasting plasma glucose; FINS, fasting serum insulin; Cr, creatinine; UA, uric acid; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; r-GT, r-glutamyl transferase; HOMA-% B, homeostasis model assessment of steady-state beta cell function; HOMA-IR, homeostasis model assessment of insulin resistance; P1,  $P$ -values for changes in variables between baseline and follow-up at 12 weeks; P2,  $P$ -values for changes in variables between baseline and follow-up at 24 weeks; P3,  $P$ -values for changes in variables between follow-up at 12 weeks and 24 weeks; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglycerides; TC, total cholesterol; HbA<sub>1c</sub>, glycosylated hemoglobin.

**Table 3** Correlations between plasma apelin levels and selected anthropometric and metabolic variables

	<i>r</i>	<i>P</i> -value
Age (years)	-0.092	0.669
BMI (kg/m <sup>2</sup> )	0.058	0.793
Waist (cm)	0.082	0.811
WHR	0.219	0.518
Systolic BP (mmHg)	0.228	0.295
Diastolic BP (mmHg)	0.179	0.413
HbA <sub>1c</sub> (%)	-0.241	0.268
TC (mmol/L)	0.016	0.943
TG (mmol/L)	0.169	0.441
HDL-C (mmol/L)	0.309	0.151
LDL-C (mmol/L)	-0.070	0.750
HOMA-% B	0.249	0.276
HOMA-IR	0.116	0.608

**Abbreviations:** BP, blood pressure; BMI, body mass index; WHR, waist to hip ratio; HbA<sub>1c</sub>, glycosylated hemoglobin; TG, triglycerides; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol; HOMA-% B, homeostasis model assessment of steady-state beta cell function; HOMA-IR, homeostasis model assessment of insulin resistance.

(GLP-1), thereby enhancing their biological actions.<sup>17</sup> Recent research suggests an interplay between apelin and GLP-1.<sup>18</sup> GLP-1 mediates the enteric and/or systemic action of apelin, and apelin also stimulates the secretion of the incretin GLP-1 both in vitro and in vivo. Watzet et al suggested that apelin might modulate digestive functions, food intake behavior, and glucose homeostasis via apelin-induced release of enteric cholecystokinins and also via incretin-releasing activity on enteric GLP-1.<sup>18</sup> This implies that the increase in plasma apelin levels associated with vildagliptin therapy might be related to increased GLP-1. The GIP receptor is expressed in adipose tissues,<sup>19</sup> and GIP plays a critical role in accumulation of fat.<sup>20</sup> These observations suggest that GIP had an effect on adipocytes, but there have been no reports published on the relationship between GIP and apelin. It is not clear that whether the vildagliptin-induced improvement in apelin levels is related to GIP. At present, the specific mechanism of the effect of vildagliptin on apelin levels is not understood, but may be related to GLP or GIP. In order to verify this hypothesis, further study of the relationship between apelin and GLP or GIP in humans is needed.

In mice, apelin was shown to act on the intestine through a luminal action to control glucose absorption.<sup>21</sup> In addition, apelin is expressed in brain regions implicated in food and water intake. One study suggested that apelin was involved in the central control of feeding.<sup>22</sup> Therefore, we speculate that inhibition of appetite by vildagliptin might be related to increased apelin levels.

We did not find any relationship between apelin and BMI in T2DM or isolated hypercholesterolemia. This implies that

the association between circulating apelin and BMI might be limited to overt obesity. Adiposity may not be a major determinant in some conditions, and different mechanisms might be involved in regulation of blood apelin concentrations. No significant relationship was found between apelin levels and insulin sensitivity in patients with T2DM in our research. It differs from previous studies probably because subjects in our research had received metformin therapy. This finding suggests that the association between insulin and apelin might not always be in the same direction under physiological or pathological conditions.

Our present study has several limitations. Because of the narrow selection criteria, the sample size was small. Hence, our data may not be representative for all subjects with T2DM. Another limitation was the use of the homeostasis model of assessment for insulin resistance index instead of the euglycemic hyperinsulinemic clamp method. The former is a less accurate method, and mainly reflects hepatic insulin sensitivity. Further larger studies are required in patients with and without vascular complications or retinopathy to investigate the role of apelin in T2DM and the effects of antidiabetic drugs on plasma levels of apelin.

## Conclusion

In conclusion, vildagliptin and metformin had a favorable effect on apelin levels and improved glycemic control. Combined treatment with metformin plus vildagliptin was superior to metformin monotherapy in terms of modifying apelin levels in patients with T2DM. This clinically important finding may be attributed to the complementary modes of action of vildagliptin and metformin. Addition of vildagliptin may be useful for patients inadequately controlled on a low dose of metformin.

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## Disclosure

The authors report no conflicts of interest in this work.

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