



Greater circulating DPP4 activity is associated with impaired flow-mediated dilatation in adults with type 2 diabetes mellitus

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Abstract *Background and aim:* Dipeptidyl peptidase 4 (DPP4) is a key enzyme involved in the regulation of the incretin system exerted by cleaving the glucagon-like peptide 1 (GLP-1); the blockage of DPP4, exerted by the antidiabetic agents DPP4-inhibitors (DPP4-I), results in greater GLP-1 concentration and improved glycaemic control. DPP4 acts also as a pro-inflammatory molecule and mediates vascular damage in experimental models. The relationship between DPP4 activity and endothelial function in diabetes has not been explored yet. Aim of this study was to investigate systemic plasma DPP4 activity in relation to endothelial function in patients with type 2 diabetes mellitus (T2DM).

Methods and results: Sixty-two T2DM individuals were recruited in our Diabetes outpatient clinics, Sapienza University, Rome, Italy. All participants underwent complete clinical work-up; endothelial function was evaluated by flow-mediated dilatation (FMD) test; plasma DPP4 activity was assessed by measuring the 7-amino-4-methylcoumarin (AMC) cleavage rate from the synthetic substrate H-glycyl-prolyl-AMC and compared with DPP4 activity measured in sixty-two age-, sex-, BMI-matched non-diabetic subjects.

Patients with T2DM had significantly higher DPP4 activity than non-diabetic individuals ($211,466 \pm 87657$ vs $158,087 \pm 60267$ nmol/min/ml, $p < 0.001$); in T2DM patients, greater DPP4 activity significantly correlated with lower FMD whereas was not associated with BMI and metabolic control. Greater systemic DPP4 activity was an independent predictor of reduced FMD after adjusting for age, gender and other confounders.

Conclusions: Circulating DPP4 activity is increased in individuals with T2DM and associated with signs of endothelial dysfunction such as impaired FMD. DPP4 may negatively affect endothelial function through mechanisms beyond glucose homeostasis and metabolic control.

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Introduction

Dipeptidyl-peptidase 4 (DPP4) is a multifunctional enzyme which acts as a serine protease receptor and costimulatory protein [1]. As a protease, DPP4 acts by cleaving N-terminal dipeptides from several substrates, including cytokines, neuropeptides, growth factors and incretins [2]. DPP4 is expressed in a membrane-bound form on the surface of several cell types, and released by proteolytic cleavage to generate the soluble form; the soluble and membrane-bound forms have analogous enzymatic activity [1]. Although DPP4 is ubiquitously expressed, main sources of this molecules are T lymphocytes, endothelial cells, smooth muscle cells, adipocytes and hepatocytes [3].

Among its substrates, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) have been widely studied for their role in modulating the postprandial secretion of insulin and glucagon – exerting the so-called *incretin effect*. Indeed, the specific inhibition of the DPP4 activity leads to increased GLP-1 concentration, improves glycaemic control in type 2 diabetes mellitus (T2DM), and is at the basis of the action of the DPP4-inhibitors (DPP4-I), a class of antidiabetic agents developed during the last decade [4].

Furthermore, DPP4 has been recently identified as a novel adipokine being produced by human adipocytes and resident adipose tissue (AT) macrophages [5,6]. DPP4 levels and activity raise in the AT and bloodstream of obese individuals and correlates with signatures of metabolic syndrome (MS), such as greater waist circumference, triglycerides and insulin-resistance [7]. In immune cells, DPP4 promotes T-cell activation and differentiation, modulation of chemokines release and tissue remodelling [8], and its expression is stimulated by inflammatory mediators such as Interferon α , β and γ [9], and IL-12 [10].

Hyperglycaemia and hypoxia are major triggers for DPP4 expression and secretion in the bloodstream by vascular cells [11–13], likely contributing to the increased plasma DPP4 levels detected in patients with T2DM [14]. Moreover, DPP4 itself has a direct effect on human vascular smooth muscle cells, stimulating inflammation and proliferative responses [15] and so participating to the complex mechanisms which lead to endothelial activation and dysfunction.

In animal models exposed to high fat diet and chronic stress, circulating DPP4 levels are increased and associated with low GLP1 and adiponectin, higher levels of inflammatory markers and greater atherosclerotic plaque growth. In these models, treatment with DPP4-I was effective in reducing stress-related atherosclerotic formation [16]. In humans, greater circulating DPP4 activity predicts the development of prediabetes and T2DM [17], atherosclerosis [18,19] and systemic hypertension [20]. Furthermore, da Silva Júnior WS and collaborators recently demonstrated the association between DPP4 activity, inflammation and microvascular damage in overweight individuals without diabetes [21].

Despite the experimental evidence on the involvement of DPP4 in vascular endothelial function and

atherosclerosis, no information is yet available on circulating DPP4 activity in relation to initial endothelial impairment in dysmetabolic conditions. Therefore, aim of this study was to investigate the association between plasma DPP4 activity and endothelial function, measured by the flow mediated dilatation (FMD) of the brachial artery in individuals with T2DM.

Methods

Population

For this study, we recruited sixty-two consecutive patients with T2DM referring to our outpatient clinics at Sapienza University of Rome, Italy, for diabetes care and meeting the following inclusion criteria: male or female subjects between 25 and 70 years of age; diagnosis of T2DM according to the American Diabetes Association 2009 criteria [22], acceptance of written informed consent. Exclusion criteria were: current/recent history of cancer, chronic terminal kidney disease, hepatic failure and/or heart failure ≥ 3 according to the New York Heart Association (NYHA) functional classification. In order to explore circulating DPP4 activity in relation to the diagnosis of T2DM, plasma DPP4 activity was also measured in sixty-two non-T2DM subjects referring to the same outpatient clinics for metabolic evaluations, and comparable for age and sex with the T2DM cohort.

All study participants underwent complete work-up including medical history and physical examination; the history of major cardiovascular (CV) events, such as myocardial infarction, stroke and peripheral arterial disease, has been specifically recorded.

Weight and height were measured with individuals wearing light clothing and no shoes; the body mass index (BMI, kg/m^2) was calculated as weight in kilograms divided by the square of the height in meters. Waist circumference (cm) was measured midway between the 12th rib and the iliac crest. Systolic and diastolic blood pressure (SBP, DBP; mmHg) were measured after five minutes resting by using an electronic blood pressure recorder with the subject sitting in the upright position.

Laboratory measurements

Overnight fasting blood sampling was performed for routine biochemistry and metabolic characterization: fasting blood glucose (FBG, mg/dl), glycosylated hemoglobin (HbA1c, % – mmol/mol), total cholesterol (mg/dl), high-density lipoprotein cholesterol (HDL, mg/dl), triglycerides (mg/dl), aspartate aminotransferase (AST, IU/l), alanine aminotransferase (ALT, IU/l), gamma-glutamyl transpeptidase (γ -GT, IU/l) and C-reactive protein (CRP, mg/dl) were measured by standard laboratory methods. Low-density lipoprotein cholesterol (LDL, mg/dl) value was calculated using Friedwald formula. Fasting blood insulin (FBI, $\mu\text{U}/\text{l}$) was assessed by radio-immuno-assay (PANTEC s.r.l., Italy; intra- and inter-assay coefficients of variation

<5%) and then the homeostasis model assessment of insulin resistance (HOMA-IR) and insulin secretion (HOMA- β) were calculated. Plasma concentrations of DPP-4 were quantified by sandwich ELISA (R&D Systems, Minneapolis, MN) according to the manufacturer's instructions.

In accordance to Matheussen et al. [23], circulating DPP4 activity was assessed by measuring the 7-amino-4-methylcoumarin (AMC) cleavage rate from the synthetic substrate H-glycyl-prolyl-AMC (H-Gly-Pro-AMC; Bio-Vision, San Francisco, California, USA, nmol/min/ml) on plasma frozen immediately after separation and stored at -25°C for few weeks. To attribute the Gly-Pro liberating activity solely to DPP4 and not to other members of DPP4 family, a parallel set of samples was incubated with the selective DPP4 inhibitor, sitagliptin, at a final concentration of 10 μM . For each sample, DPP4 activity was then calculated based on the residual fluorescence obtained by subtracting the fluorescence of inhibited sample from those of non-inhibited sample.

Cardiovascular assessments

The presence of signs of vascular dysfunction and atherosclerosis was investigated in all the T2DM patients and included the measurement of endothelial dependent Flow-Mediated Dilatation (FMD), Ankle-Brachial Index (ABI) and Intima-Media Thickness (IMT).

FMD was assessed at the level of brachial artery by mean of a high-definition linear array transducer ultrasound system (Siemens) equipped with electronic calipers, vascular software for two-dimensional imaging, color and spectral Doppler, and internal electrocardiogram; FMD was expressed as a change in post-stimulus diameter (percentage of the baseline diameter). The ABI was calculated as the ratio of ankle and brachial SBP measured separately for the right and left sides, then an average value was calculated for statistical purposes; the IMT was measured by carotid longitudinal ultrasonography (US) one centimeter proximally to the carotid bulb.

When performing the metabolic profiling, we also investigated the presence of fatty liver by abdomen US (Esaote, Genoa, Italy) conducted by the same operator blinded to laboratory values. Liver steatosis was defined on the basis of abnormally intense, high-level echoes arising from the hepatic parenchyma, liver-kidney difference in echo amplitude, echo penetration into the deep portion of the liver, and clarity of liver blood vessel structure [24]. Thus, non-alcoholic fatty liver disease (NAFLD) was diagnosed in presence of hepatic steatosis and no other causes of liver disease or steatosis [25]. Metabolic syndrome (MS) was diagnosed by the modified National Cholesterol Education Program Adult Treatment Panel III criteria [26].

Statistics

SPSS version 23 was used to perform statistical analyses. Continuous variables are reported as the mean \pm standard deviation (SD), and categorical variables as percentages. Student's T-test for continuous variables and χ^2 test for

categorical variables were used to compare mean values between two independent groups, as appropriate; skewed variables underwent natural logarithmic transformations before performing the analyses. Correlations between parameters were explored by Pearson's coefficient for continuous variables, Spearman's coefficient for categorical ones and partial correlation analyses. The multivariate linear regression analysis was performed to identify independent determinants of FMD, as considered as a continuous variable, in a sex- and age-forced model including variables significantly associated at the bivariate analysis.

This is an exploratory investigation since, at the best of our knowledge, no study has investigated so far the circulating DPP4 activity in relation to the endothelial function in patients with T2DM. Therefore, we performed a *post-hoc* sample size calculation taking into account the mean differences observed in the primary study outcome, represented by the FMD value, between subjects belonging to the lowest and highest quartiles of plasma DPP4 activity. Thus, we obtained that twenty-eight patients per subgroup would have been enough to reach the statistical significance with power = 90% and α error = 0.01. For all the above, two-sided p-value <0.05 was considered statistically significant, with 95% confidence interval (95%).

The study protocol was reviewed and approved by the local Ethics Committee and the study was conducted in conformance with the Helsinki Declaration. Written informed consent was obtained from all patients before the study procedures.

Results

Plasma DPP4 activity was significantly higher in T2DM subjects compared to non-diabetic individuals ($211,466 \pm 87,657$ nmol/min/ml vs $158,087 \pm 60,267$ nmol/min/ml, $p < 0.001$). Clinical characteristics of the entire study population, and according to the presence or absence of T2DM, are shown in Tables 1a and b. In order to explore the possible influence of DPP4-I treatment on circulating DPP4 activity and vascular function, we also included, in the T2DM cohort, subjects treated with sitagliptin 100 mg once a day for >12 months at the time of study recruitment; these patients had lower DPP4 activity than T2DM individuals treated with other antidiabetic treatments ($216,057 \pm 64,384$ nmol/min/ml vs $255,109 \pm 64,196$ nmol/min/ml, $p = 0.04$).

Circulating DPP4 activity was positively associated with greater body adiposity, as expressed by greater BMI and waist circumference, blood transaminases, presence of T2DM, MS and NAFLD (Table 2). In patients with T2DM, DPP4 activity correlated with reduced FMD ($r = -0.37$, $p = 0.012$) whereas was not associated with values of IMT ($r = -0.073$, $p = 0.63$) and ABI ($r = 0.03$, $p = 0.86$). Moreover, no correlation was found between plasma DPP4 activity, diabetes' duration, glycaemic control or treatment with DPP4-I (Table 3).

Furthermore, we measured circulating DPP4 levels in relation to both DPP4 activity and clinical parameters of

Table 1a Clinical and biochemical characteristics of study population according to the presence of T2DM.

Parameter	T2DM (n = 62)	Controls (n = 62)	P-value
Age (years)	52.4 ± 9.5	50.3 ± 10.3	0.301
Gender (%M)	66.2%	46.2%	0.060 ^a
BMI (kg/m ²)	30.3 ± 4.2	25.8 ± 4.3	<0.001
Waist Circumference (cm)	104.3 ± 12.2	88.9 ± 19.9	0.002
SBP (mmHg)	130.3 ± 16.1	120.1 ± 15.5	0.005
DBP (mmHg)	80.9 ± 9.7	77.5 ± 8.5	0.105
FBG (mg/dl)	134.1 ± 38.5	94.32 ± 14.3	<0.001
FBI (μU/l)	15.8 ± 12.8	17.4 ± 9.8	0.55
Serum creatinine (mg/dl)	0.87 ± 0.24	0.76 ± 0.17	0.033
Total - Cholesterol (mg/dl)	177.4 ± 37.8	201.2 ± 33.6	0.002
HDL- Cholesterol (mg/dl)	50.0 ± 14.9	56.1 ± 18.1	0.076
LDL - Cholesterol (mg/dl)	100.5 ± 36.5	118.8 ± 39.1	0.026
Triglycerides (mg/dl)	140.5 ± 74.7	110.0 ± 68.9	0.045
AST (IU/l)	23.4 ± 11.8	19.6 ± 5.6	0.087
ALT (IU/l)	31.5 ± 20.0	21.8 ± 10.4	0.011
γ-GT (IU/l)	40.7 ± 50.4	19.7 ± 12.1	0.002
HOMA-IR	5.1 ± 4.6	3.9 ± 2.0	0.354
HOMA-β%	105.7 ± 89.5	274.0 ± 228.3	0.017

Student's T test.

Values are expressed by mean ± SD, range or percentage, as appropriate.

BMI body mass index, SBP systolic blood pressure, DBP diastolic blood pressure, FBG fasting blood glucose, FBI fasting blood insulin, HDL-cholesterol high-density lipoprotein cholesterol, LDL-cholesterol low-density lipoprotein cholesterol, AST aspartate aminotransferase, ALT alanine aminotransferase, γ-GT gamma-glutamyl transpeptidase, HOMA-IR homeostasis model assessments of insulin resistance, HOMA-β% homeostasis model assessments of insulin secretion.

^a Chi-square test.

Table 1b Clinical characteristics of patients with T2DM (n = 62).

Parameter	Value
Disease duration (mean ± SD) (years)	7.4 ± 7
HbA1c (% - mmol/mol)	6.6 ± 1.0–49
Prevalence of Obesity (%)	37.5%
Prevalence of MS (%)	78.5%
Prevalence of NAFLD (%)	61%
Prevalence of CV events (%)	4.8%
FMD (mean ± SD) (%)	4.88 ± 4.02
IMT (mean ± SD) (mm)	0.87 ± 0.22
ABI (mean ± SD)	1.09 ± 0.15
Insulin therapy %	17%
Antidiabetic therapy %	
Biguanide	56.3%
Sulphonylureas	6.3%
DPP4-I	18%
GLP1-RA	21%
Acarbose	10.4%
Use of statins %	52%
Use of antihypertensive agents %	33%

Values are expressed by mean ± SD, range or percentage, as appropriate.

HbA1c glycosylated haemoglobin, FMD Flow Mediated Dilatation, IMT intima-media thickness, ABI Ankle-Brachial index, DPP4-I dipeptidyl-peptidase IV inhibitors, GLP1-RA glucagon like peptide-1 receptor agonists.

metabolic impairment and vascular function. Plasma DPP4 levels were significantly higher in patients with T2DM than in non-diabetic controls (mean ± SD: 568.1 ± 146.6 ng/ml vs 447.6 ± 128.9 ng/ml, $p < 0.001$). Circulating DPP4 concentration positively correlated with plasma DPP4 activity ($r = 0.38$, $p = 0.023$), increased DBP ($r = 0.31$, $p = 0.013$), transaminases (AST: $r = 0.29$, $p = 0.018$; ALT: $r = 0.29$, $p = 0.019$), FBG ($r = 0.24$, $p = 0.04$) and HOMA-IR ($r = 0.38$, $p = 0.009$), whereas no association was found between DPP4 concentration and FMD ($r = 0.02$, $p = 0.90$), IMT ($r = -0.26$, $p = 0.12$) or ABI ($r = -0.11$, $p = 0.50$) in T2DM individuals.

In consideration of the variability of diabetes' duration in the T2DM subgroup, the DPP4 activity, DPP4 concentration and their association with vascular function were investigated also according to this parameter. Thus, we found that circulating DPP4 activity, DPP4 concentration and FMD value were not different between patients with shorter or longer T2DM duration, as expressed by $<$ or ≥ 7 years from T2DM onset (subgroup with T2DM < 7 years = mean ± SD DPP4 activity: 243,944.9 ± 57,389.4 nmol/min/ml; DPP4 concentration: 568.7 ± 109.2 ng/ml; FMD: 4.4 ± 3.6% vs subgroup with T2DM ≥ 7 years = mean ± SD DPP4 activity: 249,260.1 ± 77,286.2 nmol/min/ml; DPP4 concentration: 571 ± 195.5 ng/ml; FMD: 4.4 ± 3.6%; $p = 0.79$, $p = 0.96$, $p = 0.39$, respectively). Moreover, partial correlation analyses adjusted for diabetes' duration (years, continuous variable) were performed, confirming the presence of a negative association between DPP4 activity and FMD independently from diabetes' duration ($r = -0.33$, $p = 0.031$).

In order to explore clinical determinants of impaired FMD, we conducted further analyses showing that reduced FMD significantly correlated with greater carotid IMT ($r = -0.32$, $p = 0.03$), presence of systemic hypertension ($r = -0.30$, $p = 0.019$) and the presence of an unfavourable lipid profile, as expressed by lower HDL ($r = -0.32$, $p = 0.03$) and higher triglycerides levels ($r = -0.27$, $p = 0.036$). No association was found between FMD and use of DPP4-I ($r = -0.02$, $p = 0.91$).

In the multivariate linear regression analysis, greater circulating DPP4 activity represented an independent determinant of impaired FMD independently from possible confounders, such as glycaemic control, systemic hypertension, lipid profile and systemic inflammation ($R^2 = 0.51$, standardized $\beta = -0.43$, $p = 0.005$) (Table 4).

Discussion

The main finding of this study is the presence of an independent association between higher plasma DPP4 activity and reduced FMD in T2DM patients, identifying DPP4 activity as an early marker of endothelium dysfunction in presence of diabetes. This is the first study investigating DPP4 activity in relation to the FMD, a well-

Table 2 DPP4 activity- Entire study population (n = 124).

Parameter	Correlation coefficient	P- value
Age (years)	0.33	0.727
Sex (M/F)	0.18	0.24*
BMI (kg/m ²)	0.22	0.023
Waist Circumference (cm)	0.22	0.034
SBP (mmHg)	0.13	0.177
DBP (mmHg)	0.15	0.125
Serum creatinine (mg/dl)	0.02	0.860
Total - Cholesterol (mg/dl)	0.09	0.322
HDL- Cholesterol (mg/dl)	-0.01	0.993
LDL - Cholesterol (mg/dl)	0.09	0.367
Triglycerides (mg/dl)	0.13	0.184
FBG (mg/dl)	0.07	0.433
HbA1c (%)	-0.21	0.101
FBI (μU/l)	-0.05	0.64
HOMA-IR	-0.06	0.58
HOMA-β%	-0.18	0.13
AST (IU/l)	0.28	0.003
ALT (IU/l)	0.20	0.035
γ-GT (IU/l)	0.44	<0.001
CRP (mg/dl)	0.15	0.277
T2DM (yes/no)	0.33	<0.001*
MS (yes/no)	0.20	0.029*
NAFLD (yes/no)	0.22	0.02*

Bivariate correlation analyses (Pearson's coefficient, *Spearman's coefficient, DPP4 activity is considered as a continue variable). BMI body mass index, SBP systolic blood pressure, DBP diastolic blood pressure, HDL-cholesterol high-density lipoprotein cholesterol, LDL-cholesterol low-density lipoprotein cholesterol, FBG fasting blood glucose, HbA1c glycosylated haemoglobin, FBI fasting blood insulin, HOMA-IR homeostasis model assessments of insulin resistance, HOMA-β% homeostasis model assessments of insulin secretion, AST Aspartate aminotransferase, ALT alanine aminotransferase, γ-GT gamma-glutamyl transpeptidase, C- reactive protein, T2DM type 2 diabetes mellitus, MS metabolic syndrome, NAFLD non-alcoholic fatty liver disease.

established surrogate marker of endothelial dysfunction and a predictor of future cardiovascular events [27].

Vascular cells represent the main source of circulating DPP4 in animal models [1]; moreover, in ApoE deficient mice exposed to chronic stress and high fat diet, increased plasma DPP4 levels were associated with systemic pro-inflammatory state and aberrant atherosclerotic plaque growth [16]. Wronkowitz and collaborators [15] demonstrated that soluble DPP4 induces inflammatory, stress-induced and proliferative pathways in human vascular smooth muscle cells, leading to pro-atherogenic alterations [15], whereas, in humans with T2DM greater plasma DPP4 activity was associated with carotid atherosclerosis [19]. Since the soluble DPP4 is nowadays considered as an adipokine contributing to metabolic and cardiovascular diseases [15,28], in our study we also measured circulating DPP4 concentration in relation to both DPP4 activity and clinical parameters of metabolic impairment and vascular function. Thus, we demonstrated the existence of a tight correlation between soluble DPP4 levels and its enzymatic activity. However, although greater plasma DPP4 concentration was associated with features of impaired metabolism, such as presence of T2DM, worse glycaemic control, insulin resistance and blood hypertension, no association was found between circulating DPP4 levels and markers of

Table 3 DPP4 activity- T2DM cohort (n = 62).

Parameter	Correlation coefficient	P- value
Age (years)	-0.03	0.831
Sex (M/F)	-0.10	0.960*
BMI (kg/m ²)	0.00	0.981
Waist Circumference (cm)	0.05	0.696
SBP (mmHg)	0.02	0.858
DBP (mmHg)	0.02	0.878
Serum creatinine (mg/dl)	0.18	0.160
Total - Cholesterol (mg/dl)	0.17	0.197
HDL- Cholesterol (mg/dl)	0.16	0.229
LDL - Cholesterol (mg/dl)	0.07	0.567
Triglycerides (mg/dl)	0.11	0.390
FBG (mg/dl)	0.02	0.857
FBI (μU/l)	0.18	0.26
HbA1c (%)	-0.47	0.721
AST (IU/l)	0.40	0.002
ALT (IU/l)	0.20	0.112
γ-GT (IU/l)	0.50	<0.001
CRP (mg/dl)	0.15	0.277
HOMA-IR	0.12	0.45
HOMA-β%	0.25	0.12
Systemic hypertension (yes/no)	0.34	0.801*
NAFLD (yes/no)	0.27	0.044*
MS (yes/no)	-0.02	0.860*
FMD	-0.37	0.012
IMT	-0.07	0.63
ABI	0.03	0.86
DPP4-I treatment	-0.15	0.19

Bivariate correlation analyses (Pearson's coefficient, *Spearman's coefficient, DPP4 activity is considered as a continue variable). BMI body mass index, SBP systolic blood pressure, DBP diastolic blood pressure, HDL-cholesterol high-density lipoprotein cholesterol, LDL-cholesterol low-density lipoprotein cholesterol, FBG fasting blood glucose, FBI fasting blood insulin, HbA1c glycosylated haemoglobin, AST Aspartate aminotransferase, ALT alanine aminotransferase, γ-GT gamma-glutamyl transpeptidase, C- reactive protein, HOMA-IR homeostasis model assessments of insulin resistance, HOMA-β% homeostasis model assessments of insulin secretion, NAFLD non-alcoholic fatty liver disease, MS metabolic syndrome, FMD Flow Mediated Dilatation, IMT intima-media thickness, ABI Ankle-Brachial index, DPP4-I dipeptidyl-peptidase IV inhibitors.

vascular function. Thus, in our study, the enzymatic activity of the DPP4, and not its soluble fraction in the bloodstream, represents a marker of endothelial dysfunction in the course of diabetes.

The pathophysiological processes underlying the association between increased DPP4 activity and atherosclerosis development are not fully elucidated. In condition of greater DPP4 activity, reduced GLP-1 levels may contribute to vascular damage, leading to loss of GLP-1 cardio-protective effects and hyperglycaemia [29]. In turn, hyperglycaemia itself increases the oxidative stress and leads to endothelial dysfunction and atherosclerosis by the production of advanced glycation end products (AGEs), which impair the function and integrity of many proteins [30]. Moreover, it has been demonstrated that circulating levels of AGEs positively correlated with plasma DPP4 levels [31] and the cross-talk between these two molecules may participate to the development of vascular complications in T2DM patients [32].

Table 4 Multivariate linear regression analysis. FMD is the dependent variable.

Parameter	Unstandardized coefficients		Standardized coefficients		
	β	Standard deviation error	β	<i>t</i>	<i>p</i> -value
Constant	−7673	5.770		−1.330	0.193
DPP4 activity	0	0	−0.426	−3.053	0.005
Age	0.058	0.060	0.137	0.960	0.344
Gender	0.336	1.193	0.040	0.282	0.780
TG	−0.003	0.008	−0.051	−0.325	0.747
HDL	0.099	0.042	0.406	2.376	0.024
Hb1Ac	1.1380	0.598	0.269	1.904	0.066
CPR	0.121	0.125	0.145	0.974	0.338
BMI	0.100	0.139	0.112	0.724	0.475
Hypertension	−1.138	1.202	−0.199	−1.408	0.169

$R^2 = 0.51$.

TG triglycerides, HDL-cholesterol high-density lipoprotein cholesterol, HbA1c glycosylated haemoglobin, CRP C-reactive protein, BMI Body Mass Index.

Conversely, DPP4 may also exert negative effects on vessels in a *GLP-1 independent* way by cleaving the stromal cell-derived factor 1 α (SDF-1 α), a chemokine implicated in the migration and proliferation of many cell types, such as endothelial progenitor cells (EPCs) which are recruited in areas of vascular damage [33–35]. Thus, the inhibition of SDF-1 α exerted by DPP4 may impair mechanisms of vascular repair, finally leading to accelerated atherosclerosis.

Although experimental data suggest a major role of DPP4 in mediating the processes behind atherosclerosis, little evidence is available on circulating DPP4 activity and vascular function in humans. Recently, da Silva Júnior WS and collaborators demonstrated, in overweight normoglycemic or prediabetic individuals, the association between constitutive DPP4 activity, markers of inflammation and microvascular damage, assessed as skin microvascular blood flow and vasomotion [21]. Moreover, Zheng TP and collaborators showed that in T2DM patients greater DPP4 activity correlates with the presence of carotid atherosclerosis, independently from systemic inflammation and insulin resistance [19].

The effects of DPP4-I treatment in ameliorating vascular function in T2DM was investigated in a number of clinical trials showing contrasting results [36–47]. In particular, some studies succeeded in demonstrating FMD amelioration, IMT stabilization and reduced CV disease incidence in T2DM patients treated with DPP4-I in comparison to placebo or standard of care [36–42], whereas other investigations found no significant effects [43–47].

A possible explanation for these contrasting results may be represented by differences in study population, i.e. in T2DM patients with advanced atherosclerosis and history of major CV diseases, DPP4 inhibition may not be sufficient for reversing endothelium impairment. Moreover, longer diabetes duration and worst glycaemic control - before and after treatment - likely identify *no-responder* population and may influence the outcomes of DPP4 inhibition. In addition, the inhibitory activity and DPP4 selectivity of molecules belonging to the DPP4-I class may be slightly different, and these differences may likely lead to dissimilar systemic effects on treated individuals [48].

Our investigation has been conducted on T2DM patients in good glycaemic control, with low prevalence of CV disease and no or mild IMT impairment, thus not representing a population at high risk of major CV complications. Therefore, in such patients with no evidence of clinical atherosclerosis, impaired FMD may reveal initial and subclinical alteration of arteries relaxation in response to increased shear stress, the so-called *endothelium dysfunction*, which represents the first step in the development of atherosclerosis. In our study, FMD reduction and greater DPP4 activity are strongly associated, independently from traditional risk factors of vascular damage, such as systemic hypertension, poor glycaemic control and impaired lipid profile. Circulating DPP4 activity was also associated with the presence of NAFLD, as previously shown in experimental models and humans [49,50].

Plasma DPP4 activity was greater in T2DM than non-diabetic individuals, as reported in previous investigations [14,51,52] and weakly lower DPP4 activity was observed in DPP4-I treated patients compared to T2DM subjects undergoing other antidiabetic therapies. However, no independent association was found between DPP4-I use and fasting circulating DPP4 activity and no relationship was shown between treatment with DPP4-I and indexes of vascular function.

The lack of data on the incretin system is a major limitation of this study since. However, this study has been designed to investigate systemic DPP4 activity in relation to subclinical vascular impairment, as measured by FMD, regardless its role as a cleavage enzyme regulating GLP1 concentration and, thus, the incretin activity. Despite the cross-sectional design of this study that does not allow to establish a causal nexus, the overall results of this study point towards the possible role of circulating DPP4 activity as a novel marker of subclinical vascular damage in metabolic diseases.

In conclusion, this is the first study demonstrating the association between systemic DPP4 activity and signs of early atherosclerosis in T2DM patients, independently from traditional CV risk factors such as metabolic control and systemic hypertension. Further studies are warranted for investigating endothelial function in relation to DPP4

activity in populations other than T2DM individuals and specifically designed for exploring effects of early DPP4-I treatment on subclinical vascular parameters.

Disclosures

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