Original Research

ADMA (asymmetric dimethylarginine) and angiogenic potential in patients with type 2 diabetes and prediabetes

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Impact statement

Our research provided new insight into the mechanisms governing vascular complications in prediabetes and type 2 diabetes. Unfortunately, most studies focus on angiogenesis markers (VEGF-A, sVEGF-R1, sVEGF-R2) and endothelial dysfunction marker (ADMA) separately. Our findings reported for the first time that endothelial damage and angiogenic potential at early stage of carbohydrate dysfunction appear in prediabetes before type 2 diabetes is diagnosed.

Abstract

Asymmetric dimethylarginine is an endogenous competitive inhibitor of nitric oxide synthase and marker of endothelial dysfunction, but the question remains as to whether asymmetric dimethylarginine is a marker of cardiovascular episodes or their independent risk factor. ADMA/DDAH (dimethylaminohydrolase) pathway regulates vascular endothelial growth factor (VEGF)-mediated angiogenesis due to its impact on the NO formation. The aim of the study was to assess the concentrations of asymmetric dimethylarginine and the angiogenic potential in the blood of subjects with type 2 diabetes (T2DM, $n = 33$) and patients with prediabetes ($n = 32$)—impaired fasting glycemia and/or impaired glucose tolerance (WHO criteria). The study found that both the prediabetes group and subjects with T2DM had significantly elevated concentrations of asymmetric dimethylarginine, significantly high levels of VEGF-A, low ratio of sVEGF-R1/VEGF-A, and sVEGF-R2/VEGF-A. This may suggest endothelial damage at early stages of carbohydrate metabolism dysfunction—before T2DM is diagnosed. Higher proangiogenic potential in prediabetes and T2DM patients than in healthy subjects, is not only the effect of an increase in VEGF-A levels, but also reduced inhibition of circulating receptors.

Keywords: Endothelial dysfunction, asymmetric dimethylarginine, type 2 diabetes mellitus, prediabetes, angiogenesis, growth factors

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Introduction

Asymmetric dimethylarginine (ADMA) is an endogenous competitive inhibitor of nitric oxide synthase (NOS). It is the result of protein L-arginine methylation mediated by protein arginine methyltransferases (PRMTs), and eliminated from the body by kidneys which are responsible for reducing plasma methylated arginine levels by ca. 10%. An important regulatory mechanism of ADMA blood levels is dimethylaminohydrolase (DDAH) which breaks down ADMA into citrulline and dimethylamine.[1] ADMA causes dysfunction of endothelium forearm arterial bed in healthy subjects.[2] It increases systemic vascular resistance and blood pressure, and leads to a reduced cardiac output.[3]

Zoccali *et al.*,[4] in the prospective study, demonstrated a correlation between the circulating concentration of ADMA and prospective cardiovascular episodes and the mortality rate in patients with acute kidney failure. The study found that elevated plasma levels of ADMA were observed in patients with normal renal function or mild kidney dysfunction and unfavorable cardiovascular risk profile including individuals with peripheral arterial occlusive disease (PAOD), hypertension, hyperlipidemia, insulin resistance, type 1 and 2 diabetes, diabetic nephropathy, hypopituitarism, and in women with early gestational diabetes.[1] In the light of clinical and laboratory observations, the question remains as to whether ADMA is the marker of

cardiovascular episodes or their independent risk factor. It turned out that the continuous infusion of ADMA to mice for four weeks resulted in microscopic changes in the ventricles of these animals, but overexpression of DDAH—an enzyme that breaks down ADMA, decreased the ADMA levels and retreated the changes in coronary vessels.[5,6]

Diabetes is a disease which affects all communities and, due to lifestyle changes for less active ones, the prevalence of this disease is increasing at concerningly greater rates. Epidemiological studies conducted in 2015–2017 revealed that 415 million people had diabetes at that time and, considering the increasing incidence rate, it is estimated that around 642 million people will suffer from diabetes in 2040, and, depending on the communities studied, this number will include 80–85% of patients with type 2 diabetes.[7]

In vitro studies have shown that hyperglycemia distorts the activity of DDAH in smooth muscle cells and endothelium, and decreases NO signaling which contributes to elevated ADMA levels in patients with diabetes.[8] Fiedler at al. reported that ADMA inhibited endothelial cell polarization, protrusion formation, and reduced focal adhesion dynamics. The authors concluded that the ADMA/DDAH pathway regulates vascular endothelial growth factor (VEGF)-mediated angiogenesis due to its impact on the NO formation.[9]

NO inhibits apoptosis, increases endothelial cell proliferation, stimulates the DNA synthesis of endothelial cells and proliferation by cGMP-dependent transcription, and therefore it is the key regulator of angiogenesis. Reducing the formation of NO, ADMA inhibits the endothelial cell proliferation and angiogenesis.[10] It has been shown that DDAH-1—an enzyme responsible for breaking down ADMA, has influence on the cyclins of endothelial cells, and inhibits the proliferation of endothelial cells and angiogenesis. However, recent studies have shown that hypoxia may be a factor that increases the expression of DDAH-1 which promotes VEGF-stimulated angiogenesis.[11]

Angiogenesis disorders are essential in the pathogenesis of diabetes. Diabetic patients have a reduced number of circulating endothelial progenitor cells (EPCs) and impaired functions of EPCs. Lower expression of VEGF-A and VEGF receptors as well as elevated concentrations of SDF-1 (stromal-derived growth factor-1), PDGF (platelet-derived growth factor), and angiopoietins were found in the myocardium of diabetic patients.[12–14]

The analysis of available literature has no references to the assessment of the ADMA concentrations associated with the angiogenic potential in the blood of patients with type 2 diabetes expressed as the ratio of proangiogenic VEGF-A and circulating receptors sVEGF-R1 and sVEGF-R2 being endogenous inhibitors of angiogenesis.

The purpose of the research study was the evaluation of the concentrations of ADMA and the angiogenic potential in the blood of subjects with type 2 diabetes and patients with prediabetes (impaired fasting glycemia and/or impaired glucose tolerance).

Materials and methods

Study design

The study group was composed of 65 patients with carbohydrate metabolism dysfunction (WHO criteria in Oral Glucose Tolerance Test) including 32 subjects with prediabetes—IFG and/or IGT—(22 F and 10 M) aged 38–78 years and 33 individuals with type 2 diabetes (T2DM, 15 F and 18 M) at the age of 40–79 years. The control group included 30 healthy subjects without carbohydrate metabolism dysfunction, with normal BMI (Body Mass Index), and non-smokers (15 F and 15 M aged 35–71 years). Table 1 displays the characteristics of the study group.

Citrate venous blood was used to define the concentrations of ADMA (*Cloud-Clone Corp®, USA*), VEGF-A, sVEGF-R1, and sVEGF-R2 (*Quantikine, R&D®, USA*) by means of the ELISA technique.

The proangiogenic potential was measured using the ratio which is the quotient of the inhibitor (sVEGF-R1 or sVEGF-R2) and the proangiogenic VEGF-A.

All study subjects were informed about the purpose of the research. The studies were authorized by the local Bioethics Commission of Ludwik Rydygier Collegium Medicum in Bydgoszcz, Nicolaus Copernicus University in Toruń, no. KB 627/2016 and were carried out in accordance with the Declaration of Helsinki. Obtaining blood samples was preceded by giving informed consent. In addition to the lack of informed consent, pregnancy, and the history of cancer, the exclusion criteria included acute cardiovascular incident during the last six months, metabolic imbalance (HbA1c $>9\%$), diabetic retinopathy, dialysis, chronic kidney disease with eGFR $<60\,mL/min/1.73\,m^2$ (the CKD-EPI), and acute and chronic inflammatory diseases. Table 2 displays the inclusion and exclusion criteria of cardiovascular symptoms.

Table 1. Clinical data concerning patients with prediabetes and type 2 diabetes.

Parameter	PREDIABETES $n=32$	T2DM $n=33$	P
Sex (F/M)	22/10	15/18	NS
Age (years)	57 ± 11	63 ± 8	NS
Duration of disease (months)	14.7 ± 14.6	72 ± 70.75	<0.001
BMI (kg/m^2)	31.1 ± 6.2	30.99 ± 6.4	NS
HbA1c (%)	5.6 ± 0.35	7 ± 1.29	<0.001
Creatinine (mg/dL)	0.85 ± 0.17	0.9 ± 0.3	NS
eGFR (according to CKD-EPI, mL/min/1.73 m^2)	84.29 ± 16	82.27 ± 20.6	NS
LDL (mg/dL)	93.9 ± 49.9	97 ± 41	NS
TG (mg/dL)	121.1 ± 57.3	158.2 ± 76.3	NS
Smoking (*n*/%)	9 (28%)	22 (67%)	<0.001
CAD (*n*/%)	6 (19%)	13 (39%)	<0.001
CerAD (*n*/%)	5 (16%)	12 (36%)	<0.001
LEAD (*n*/%)	0 (0%)	2 (6%)	<0.001
Hypertension (*n*/%)	22 (69%)	31 (94%)	<0.001

CAD: coronary artery disease; CerAD: cerebral artery disease; LEAD: lower extremity artery disease.

Table 2. The inclusion and exclusion criteria of cardiovascular symptoms.

		Inclusion criteria	Exclusion criteria
CAD	Coronary artery disease	Stable angina pectoris, past myocardial infarction, CABG, PCI/angioplasty (≥6 months), atherosclerotic plaques in coronary angiography	Unstable angina, myocardial infarction, CABG, PCI/angioplasty (<6 months)
CerAD	Cerebral artery disease	Past stroke/TIA (≥6 months), atherosclerotic plaques in ultrasound/angiography, thickening of the intima-media complex	Past stroke/TIA/thrombolytic treatment (<6 months)
LEAD	Lower extremity artery disease	Intermittent claudication, ABI ≤0.9, past PCI/angioplasty (≥6 months), atherosclerotic plaques in ultrasound/angiography	Critical/acute limb ischemia, PCI/angioplasty (<6 months)

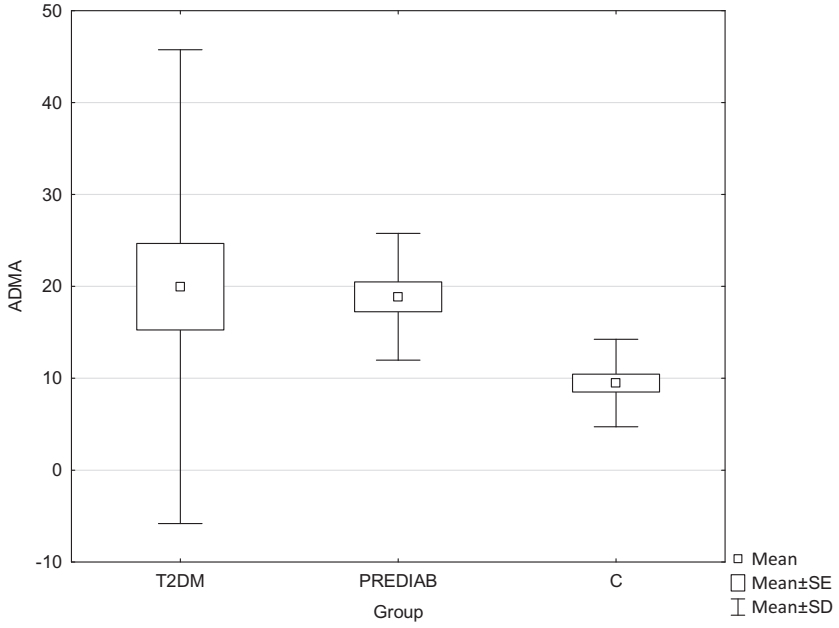

Figure 1. ADMA concentrations in the groups with T2DM and prediabetes against the control group (C).

Statistical analysis

The statistical analysis was conducted using Statistica 12.0 software (*StatSoft®, Cracow, Poland*). The compatibility of examined parameters distribution with the standard normal distribution was assessed by the W Shapiro–Wilk test. The significance level was set at $P < 0.05$.

Results

Significantly higher, twice the norm, concentrations of ADMA were found in the group of patients with T2DM against healthy subjects (19.4 ± 24.7 vs. 9.2 ± 4.8 ng/mL, $P < 0.006$) and the same levels were noticed in the prediabetes group (18.7 ± 6.7 vs. 9.2 ± 4.8 ng/mL, $P < 0.0001$). However, the differences between the groups of patients were not statistically significant (Figure 1).

VEGF-A levels were significantly (more than twice) higher in patients with T2DM and prediabetes as compared to healthy individuals (respectively 56.24 ± 49.52 and 55.82 ± 43.16 vs. 24.97 ± 18.21 pg/mL, $P = 0.0004$ and $P = 0.001$), without any significant differences between the subgroups of patients (Figure 2).

Significantly elevated levels of sVEGF-R1 and insignificantly lower concentrations of sVEGF-R2 were observed in the subjects from both study groups against the control group (Table 3).

Based on the obtained results, the ratio of sVEGF-R1/VEGF-A and sVEGF-R2/VEGF-A was determined.

The quotient of the sVEGF-R1/VEGF-A concentrations in the T2DM group was the lowest and ranged between 6.45 ± 3.88, and in the prediabetes group it was 9.10 ± 8.25, with the values of 12.41 ± 14.98 found in the control group. Despite the observed differences, the significance threshold was not found due to large statistical deviations (Figure 3).

The sVEGFR-2/VEGF-A ratio was significantly lower in the individuals with T2DM when compared to healthy subjects (211.38 ± 118.39 vs. 747.66 ± 1160.69, $P = 0.0002$), like in the prediabetes group (233.57 ± 211.07 vs. 747.66 ± 1160.69, $P = 0.0002$, Figure 4), and without any significant differences between the subgroups of patients.

The conducted assessment included correlations between ADMA levels in the blood of both groups of patients and the concentrations of VEGF-A, sVEGF-R1, and sVEGF-R2, as well as correlations between the levels

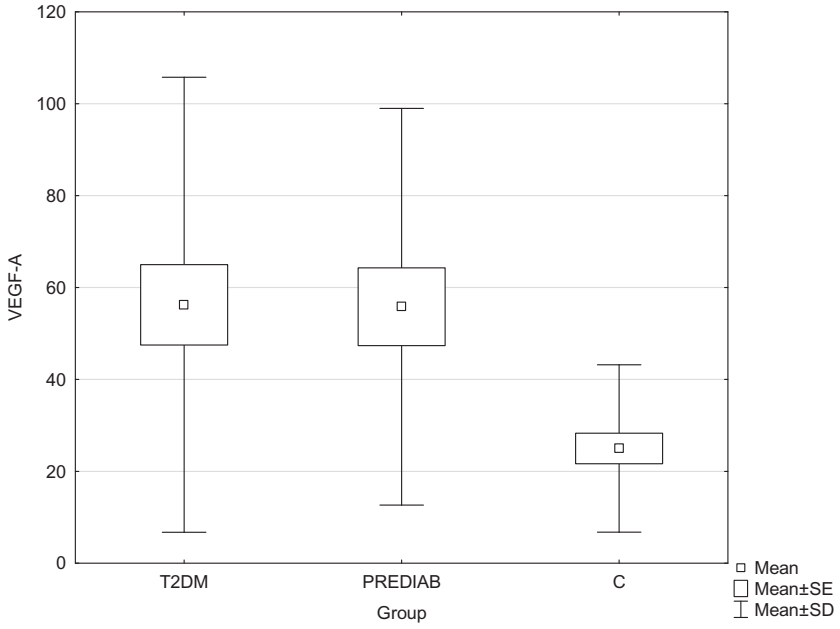

Figure 2. The concentrations of VEGF-A in the groups with T2DM and prediabetes against the control group (C).

Table 3. Concentrations of sVEGF-R1 and sVEGF-R2 in the study groups and the control group.

	T2DM ***n*** **= 33** **a**	**Prediabetes** ***n*** **= 32** **b**	**Controls** ***n*** **= 30** **c**	***P***
sVEGF-R1 [pg/mL]	269.95 ± 125.29	332.2 ± 175.1	156.45 ± 54.68	a vs. b NS **a vs. c 0.0002** **b vs. c < 0.0001**
sVEGF-R2 [pg/mL]	8258.7 ± 1764.7	8303.4 ± 1613.15	9136.13 ± 1846.89	a vs. b NS a vs. c NS b vs. c NS

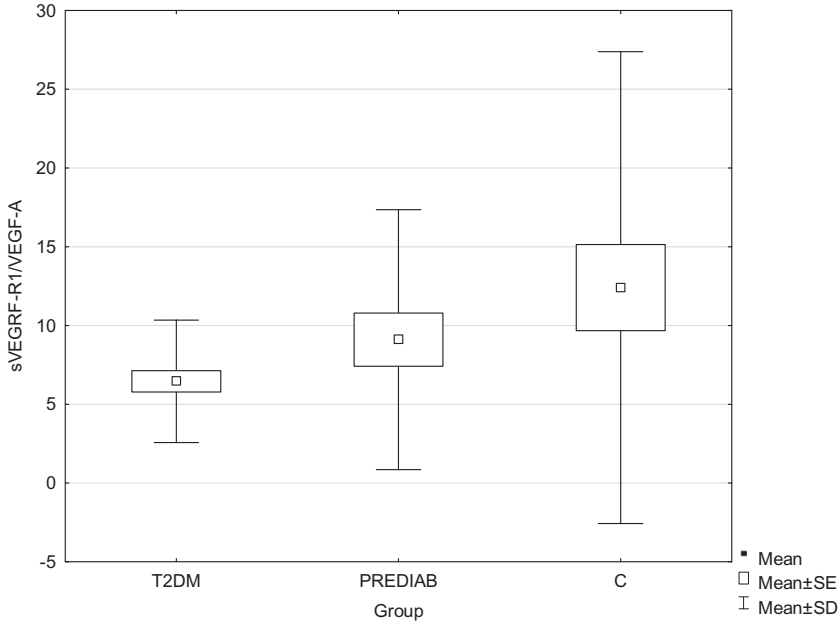

Figure 3. The sVEGFR-1/VEGF-A ratio in the T2DM and prediabetes groups vs. the control group (C).

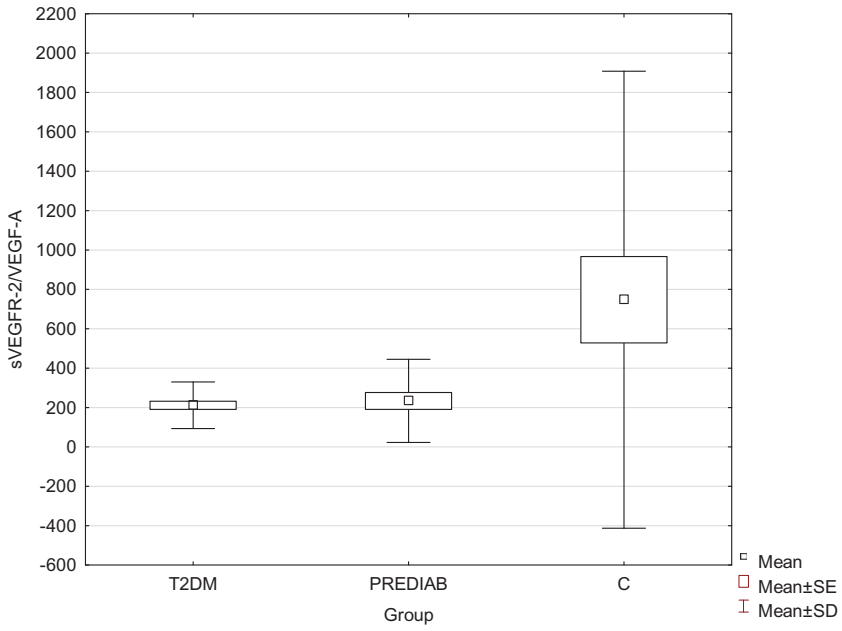

Figure 4. The sVEGFR-2/VEGF-A ratio in groups T2DM and prediabetes against the control group (C).

of ADMA and the quotients of sVEGF-R1/VEGF and sVEGF-R2/VEGF-A. No significant relationships were reported in the study subjects between the concentrations of ADMA and VEGF-A, as well as sVEGF-R1 and sVEGF-R2. A significant negative correlation was observed between the VEGF-A level and the quotient of sVEGF-R1/VEGF-A and sVEGF-R2/VEGF-A representing an angiogenesis inhibition (Table 4). A strongly negative, statistically significant correlation at the level of −0.93 was reported between VEGF-A and sVEGF-R1/VEGF-A (the inhibition potential) in the control group. The correlation between these parameters decreased to −0.78 in prediabetic patients and to −0.56 in T2DM subjects. The analysis concerning the correlation of VEGF-A and the inhibition ratio of sVEGFR-2—sVEGFR-2/VEGF-A did not reveal any changes in the correlation between the control group, individuals with prediabetes, and T2DM patients.

Table 5 presents the evaluation of relationships between the level of ADMA and the selected clinical and biochemical parameters.

The concentration of ADMA significantly correlated with age and BMI in healthy subjects. The prediabetes group showed significantly high positive correlation of ADMA and LDL, as well as negative correlation of ADMA and creatinine. ADMA negatively correlated with fasting plasma glucose (FPG) in patients with T2DM.

Table 4. The analysis of correlations between VEGF-A as well as the ratio of sVEGF-R1/VEGF-A and sVEGF-R2/VEGF-A.

	sVEGF-R1/VEGF-A		sVEGF-R2/VEGF-A		
VEGF-A	**R**	***P***	**R**	***P***	
	−0.93	<0.000001	−0.97	<0.000001	**Controls**
	−0.78	<0.000001	−0.97	<0.000001	**Prediabetes**
	−0.56	0.0009	−0.92	<0.000001	**T2DM**

Table 5. The evaluation of correlations between blood ADMA levels in prediabetes and T2DM patients and selected clinical and biochemical parameters (age, fasting plasma glucose (FPG), BMI, HbA1c, levels of LDL, TG, creatinine, and eGFR).

	ADMA		
	Prediabetes	**T2DM**	**Controls**
Age	*NS*	*NS*	R = 0.58 *P* = 0.002
FPG	*NS*	R = −0.41 *P* = 0.019	*NS*
BMI	*NS*	*NS*	R = 0.4 *P* = 0.047
HbA1c	*NS*	*NS*	–
LDL	R = 0.69 *P* = 0.028	*NS*	–
TG	*NS*	*NS*	–
Creatinine	R = −0.69 *P* = 0.015	*NS*	–
eGFR (CKD-EPI)	*NS*	*NS*	–

FPG: fasting plasma glucose.

Discussion

The study found that both the prediabetes group and subjects with T2DM had significantly elevated concentrations of ADMA and significantly high levels of VEGF-A and the low ratio of sVEGF-R1/VEGF-A and sVEGF-R2/VEGF-A.

The review of relevant literature provides only a few publications which analyze the significance of ADMA levels, particularly in the context of angiogenesis in patients with carbohydrate metabolism dysfunction, especially prediabetes.

One of the essential factors of metabolic syndrome is carbohydrate metabolism dysfunction such as prediabetes

or diabetes. The research conducted by Głowińska-Olszewska *et al.*[15] reported that metabolic syndrome, already diagnosed in adolescents, was accompanied by elevated levels of ADMA. Siervo and Bluck,[16] and Palomo *et al.*[17] reported higher levels of circulating ADMA in obese patients with metabolic syndrome. Likewise, elevated ADMA was observed in obese individuals ($BMI \geq 30\,kg/m^2$) with normal values of blood pressure.[18] This study found that average BMI was very high in subjects with T2DM ($30.99 \pm 6.4\,kg/m^2$) and prediabetes ($31.1 \pm 6.2\,kg/m^2$), which suggested common coexistence of obesity according to BMI criteria set out by the WHO, and may have significantly affected the obtained results.

Elevated concentrations of ADMA were reported in previous observations not only in patients with type 2 diabetes,[19] but also type 1 diabetes,[20] or insulin resistance.[21,22] The common denominator of these disorders is hyperglycemia (especially postprandial) which is associated with elevated levels of ADMA proven in the studies conducted by Konukoglu *et al.*[23] concerning both people suffering from prediabetes and T2DM. The influence of type 1 diabetes on ADMA levels is the object of the studies carried out by our team and will be presented in the upcoming publications. High concentrations of ADMA were also seen in women with previous gestational diabetes[24,25] and carbohydrate intolerance in pregnancy.[26] In this study, pregnancy was one of the exclusion criteria.

In this study, positive correlation of ADMA levels and age was found only in healthy subjects. Moreover, they had positive correlation between ADMA and BMI. A statistically significant strong positive correlation of ADMA and LDL existed in the prediabetes group. The study of Anderssohn *et al.* identified determinants of plasma ADMA concentrations depending on demographic and biochemical factors, and found that there was no impact of age, sex, duration of diabetes, HbA1c, total cholesterol, HDL, triglycerides, and BMI. However, a positive relationship was reported between ADMA and respectively: fasting plasma glucose, creatinine, and L-arginine.[27]

The analysis of available studies concerning carbohydrate metabolism dysfunction parameters in patients with type 2 diabetes in relation to ADMA level measurement indicates some discrepancies in various publications. This study found negative correlation of ADMA and fasting plasma glucose in the type 2 diabetes group, whereas Eliana *et al.*[28] obtained a positive relationship of ADMA and glycemia level tested 2 h after a meal. Surdacki *et al.*[29] did not report correlation between the levels of ADMA and glycemia. The study conducted by Eliana *et al.* found positive strong correlation ($R = 0.72$) between ADMA and HbA1c.[28] Positive correlation of ADMA and HbA1c was also observed by Začiragić *et al.*[30] When conducting research on 270 patients with type 2 diabetes, Hsu *et al.*[31] did not find significant correlation of ADMA and HbA1c. Can *et al.*[32] reported elevated concentrations of ADMA in the group of T2DM subjects with lower metabolic balance ($HbA1c > 6.5\%$) as compared to patients with well controlled diabetes ($HbA1c < 6.5\%$). In this study, the T2DM group had an average HbA1c percentage amounting to $7 \pm 1.29\%$, and metabolic dysfunction (HbA1c >9%) was an exclusion criterion. Therefore, a relatively good metabolic balance in our patients may have caused us not to observe a relationship between ADMA and HbA1c in this study.

Vascular complications, i.e. macro- and microangiopathy which can be associated with ADMA pathway disorders, are essential in the pathogenesis of type 2 diabetes.

Increased risk of macroangiopathic complications, connected e.g., with high concentrations of ADMA at an advanced stage of impaired glucose tolerance (IGT) was reported by Huang *et al.*[33] Protopsaltis *et al.*,[34] when studying pulse wave velocity (central arterial stiffness), reported high concentrations of ADMA already in prediabetes. This is consistent with our observations. High concentrations of ADMA can be the predicator of serious cardiovascular incidents as shown in research conducted by Hsu et al. During 5.7 years, major adverse cardiovascular events (MACE) occurred in 20.4% of 270 patients, including death due to cardiovascular diseases such as myocardial infarction or cerebral stroke, and the authors found that high initial levels of ADMA comprised an independent risk factor of these incidents.[31]

Five-year observations conducted by Konya *et al.*[35] on T2DM patients also confirmed the special role of elevated plasma ADMA as an increased cardiovascular risk factor. Higher ADMA/L-arginine ratio was already observed in patients with prediabetes and two- or three-vessel coronary artery disease.[29] An increased risk of death and/or myocardial infarction in correlation with increasing concentrations of ADMA was also observed during a 2.6-year study of 850 patients including subjects with type 2 diabetes or prediabetes.[36] Borgeraas *et al.*,[37] when observing nearly 3000 patients for five years, reported an increased risk of myocardial infarction and cardiovascular death accompanied by increasing ADMA in the population of Norway. This study reported 39% of patients with the history of CAD and 36% of patients with the history of CerAD individuals with type 2 diabetes, and in case of prediabetes subjects, this percentage was 19 and 16% respectively. During 4.5-year observations, Surdacki *et al.* found that among 80 sick male subjects without the history of diabetes, who had coronary angioplasty, 11 individuals developed DM2 during the study and 13 subjects had impaired fasting glycemia (prediabetes). A significant predictive factor was also a high initial level of ADMA.[38] In consequence of high concentrations of ADMA obtained in this study, both in patients with T2DM and prediabetes, a follow-up observation of this population could be an interesting continuation of our research.

High ADMA levels reported by Jing *et al.* were connected with the development of microvascular complications in prediabetes as well as T2DM.[39] Du et al.[40] in the literature review in their article from 2016, emphasized the occurrence of elevated ADMA levels in the development of microangiopathic complications (retinopathy, nephropathy, or neuropathy)[40] One of the factors influencing plasma ADMA is the kidney performance level—the first observations concerning the role of ADMA related to patients with acute kidney failure.[41] High concentrations of ADMA in T2DM patients were associated with a greater

incidence of the so-called major adverse renal events (MARE) in patients after coronary angioplasty—defined as death, initiating dialysis or doubling of the creatinine level.[42] In this study, mean concentrations of creatinine and eGFR (according to CKD-EPI) in T2DM and prediabetes subjects were respectively: 0.9 ± 0.3 mg/dL and 82.27 ± 20.6 mL/min/1.73 m^2, 0.85 ± 0.17 mg/dL and 84.29 ± 16 mL/min/1.73 m^2. Interestingly, correlation of ADMA and creatinine levels was significantly negative in the prediabetes group. Zobel *et al.*,[43] when studying 200 patients with T2DM and microalbuminuria (without ischemic heart disease symptoms) for 6.1 years, confirmed the role of ADMA as a predicator of increased mortality rate in these patients. Hanai *et al.*[44] considered high values of ADMA as a predicated factor of diabetic nephropathy progression in the study on 225 adult Japanese patients. Similarly, ADMA concentrations correlated with the creatinine level in the study conducted by Onat *et al.*[45] It should be highlighted that the present study was carried out on patients with eGFR > 60 mL/min/1.73 m^2, which can significantly affect the potential findings.

Pathological angiogenesis in T2DM leads to increased retinopathy and diabetic foot syndrome—in addition to elevated VEGF-A levels[46,47] and progression of Critical Limb Ischemia (CLI).[48] High concentrations of ADMA are thought to be associated with the presence and progression of diabetic retinopathy, which has been confirmed in numerous studies including Abhary et al.,[49] Malecki *et al.*,[50] Yonem *et al.*,[51] and Tasci *et al.*[52] Increased levels of VEGF-A are observed in diabetic retinopathy, which has been used in common treatment with anti-VEGF drugs (e.g. bevacizumab). This study included diabetic retinopathy in the exclusion criteria, although it seems to be a common denominator for the VEGF-A and ADMA/DDAH-2 pathway.

The role of angiogenesis in carbohydrate metabolism dysfunction has been ambiguous for many years.[53] This may be associated with unbalancing between the factors responsible for initiating and inhibiting the formation of new blood vessels and thus with disorders of physiological angiogenesis—necessary for e.g., normal wound healing,[54] or with the development of collateral circulation in patients with T2DM and concomitant PAD.[55] Advanced endothelial dysfunction identified already in newly diagnosed type 2 diabetes expressed by high concentrations of ADMA was highlighted by Sciacqua *et al.*[56] This study found that the concentrations of ADMA were high both in the T2DM group and in patients with prediabetes, which suggests endothelial dysfunction at the early stages of carbohydrate metabolism dysfunction. It should be emphasized that our studies were conducted on a group of patients with a relatively short history of T2DM (ca. 72 ± 70.75 m-cy), and in the prediabetes group the time before diagnosis was 14.7 ± 14.6 months.

Significant supplementing of vascular studies may be calculating the quotient of sVEGF-R1/VEGF-A and sVEGF-R2, i.e. the so-called angiogenic potential. The sVEGFR-2/VEGF-A ratio was significantly lower both in individuals with T2DM and prediabetes as compared to healthy subjects. Furthermore, strong negative correlation was observed between the concentration of VEGF-A and the sVEGF-R1/VEGF-A ratio (inhibition potential) in the control group. This means that among healthy individuals existed outstanding negative correlation of proangiogenic VEGF-A and the inhibitory sVEGF-R1/VEGF-A ratio depending on sVEGF-R1. Circulating sVEGF-R1 is known as a trap receptor because its inhibitory function involves binding VEGF-A and forming inactive complexes of VEGF-A-sVEGF-R1. In consequence, there is a smaller number of VEGF-A molecules in the vessel which, in turn, reduces VEGF-A availability to the fixed membrane receptor VEGF-R2 existing on the surface of endothelial cells (and being responsible for the biological effect of VEGF-A), and therefore inhibits or diminishes angiogenesis. Correlation of these parameters in patients with prediabetes and T2DM is less negative which suggests a progressive decrease in angiogenic inhibition—first in prediabetes, then in T2DM.

Limitations

Other important factors influencing the concentration of ADMA in the context of angiogenesis in T2DM patients may include physical activity[57] and pharmacotherapy, e.g. with metformin,[58–60] pioglitazone,[61] or insulin.[62,63] All these factors should be the subject of further considerations in subsequent studies on ADMA level in patients with carbohydrate metabolism dysfunction. It appears that an important link between the pathway of ADMA/DDAH-2 and VEGF-A/sVEGF-R1/R2 can be circulating endothelial progenitor cells (EPCs) whose reduced number reflects little response of marrow to the formation of these cells despite strong hypoxemia impulse in tissues.[64] Accelerated senescence of circulating endothelial cells is observed in T2DM patients, which is affected by excessive ADMA and DDAH-2 deficiency.[65] Transplantation of autologous bone marrow mononuclear cells incubated with VEGF gene improved prognosis for CLI caused by type 2 diabetes.[66] Also, so-called ADMA/SDMA/hArg paradox may be solved by the assumption that not the free acids but their precursor proteins exert biological effects in the vasculature.[67] Therefore, further extensive studies are required in this respect.

Conclusions

Elevated concentrations of ADMA and VEGF-A are not only in the blood of type 2 diabetes patients, but also in individuals with prediabetes and are twice as high compared to healthy subjects. This may suggest endothelial damage at early stages of carbohydrate metabolism dysfunction—before type 2 diabetes is diagnosed.

Higher proangiogenic potential in prediabetes and T2DM patients than in healthy subjects, is not only the effect of an increase in VEGF-A levels, but also reduced inhibition of circulating receptors.

Authors' contributions: All authors contributed significantly to conception and design or analysis and interpretation of data and drafting of the manuscript intellectual content.

DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

ETHICAL APPROVAL

The studies were authorized by the local Bioethics Commission of Ludwik Rydygier Collegium Medicum in Bydgoszcz, Nicolaus Copernicus University in Toruń, no. KB 627/2016 and were carried out in accordance with the Declaration of Helsinki.

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