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ORIGINAL ARTICLE

Differential associations of circulating asymmetric dimethylarginine and cell adhesion molecules with metformin use in patients with type 2 diabetes mellitus and stable coronary artery disease

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Abstract Metformin, the drug of first choice in type 2 diabetes mellitus (T2DM), reduces cardiovascular (CV) morbidity and mortality in part independently of improved glycemic control and changes in traditional risk factors. However, there are discordant reports on the effects of metformin on endothelial function in T2DM. Our aim was to compare biochemical endothelial markers in patients with stable coronary artery disease (CAD) and T2DM stratified by metformin use. We studied 70 patients (29 women, age 68 ± 9 years) with established T2DM referred for elective coronary angiography owing to stable angina who were receiving a standard CV medication and metformin or other oral antidiabetic drugs. Exclusion criteria included heart failure and other relevant coexistent disorders. Biochemical indices of endothelial dysfunction and activation at admission were compared according to metformin use for at least 1 year prior to index hospitalization. Clinical characteristics were similar in patients receiving metformin ($n = 40$) vs. those on other oral antidiabetic agents ($n = 30$). Plasma soluble vascular cell adhesion molecule-1 (sVCAM-1) was lower (553 ± 148 vs. 668 ± 170 μg/L, $P = 0.004$) and asymmetric dimethylarginine (ADMA) higher (0.53 ± 0.09 vs. 0.48 ± 0.08 μM, $P = 0.01$) in subjects on metformin, which was maintained in multivariate analysis. Symmetric dimethylarginine, intercellular adhesion molecule-1, monocyte chemotactic protein-1 and E-selectin did not differ across the groups. The results were substantially unchanged after exclusion of insulin users. Thus, metformin use appears differentially associated with sVCAM-1 and ADMA in patients with T2DM and stable CAD. Whether this observation may reflect different prognostic effects of these endothelial markers in diabetes remains to be studied.

Keywords Adhesion molecules · Asymmetric dimethylarginine · Coronary artery disease · Metformin · Type 2 diabetes mellitus

Abbreviations

ACEI	Angiotensin-converting enzyme inhibitors
ADMA	Asymmetric dimethylarginine
ANCOVA	Analysis of covariance
CAD	Coronary artery disease
CV	Cardiovascular
DDAH	Dimethylarginine dimethylaminohydrolase
eGFR	Estimated glomerular filtration rate
ELISA	Enzyme-linked immunosorbent assay
eNOS	Endothelial nitric oxide synthase
HbA1c	Glycated hemoglobin
HDL	High-density lipoproteins
LDL	Low-density lipoproteins
MCP-1	Monocyte chemotactic protein-1
NO	Nitric oxide
SDMA	Symmetric dimethylarginine
sICAM-1	Soluble intercellular adhesion molecule-1
sVCAM-1	Soluble vascular cell adhesion molecule-1
T2DM	Type 2 diabetes mellitus
vWF	von Willebrand factor

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Introduction

According to a position statement of the American Diabetes Association and the European Association for the Study of Diabetes, metformin is the optimal first-line drug in type 2 diabetes mellitus (T2DM) (Inzucchi et al. 2012). The ability of metformin to reduce the risk of adverse cardiovascular (CV) events and overall mortality was in part independent of improved glycemic control and changes in traditional CV risk factors associated with metformin therapy (UK Prospective Diabetes Study (UKPDS) Group 1998; Holman et al. 2008; Roussel et al. 2010; Roumie et al. 2012; Scheen and Paquot 2013).

However, there are inconsistent reports on the ability of metformin to correct endothelial dysfunction, a well-recognized antecedent of adverse CV events. Despite the fact that almost 20 years ago a metformin-induced improvement of hemodynamic and rheological responses to L-arginine in patients with T2DM was demonstrated (Marfella et al. 1996), contradictory results were obtained in later studies, irrespective of whether endothelium-dependent vasodilatory responses (Mather et al. 2001; Abbink et al. 2002; Natali et al. 2004; Naka et al. 2012; Wu et al. 2014) or biochemical markers (de Jager et al. 2005; Kautzky-Willer et al. 2006; Skrha et al. 2007; Lund et al. 2008; Fidan et al. 2011) were used to estimate endothelial function. Of note, in a recent randomized study a 3-day treatment with metformin pretreatment did not protect against endothelial ischemia–reperfusion injury within the brachial artery in healthy middle-aged subjects (El Messaoudi et al. 2014).

Furthermore, discordant results were obtained in the only two studies (Asagami et al. 2002; Lund et al. 2008) that investigated the influence of metformin in T2DM on the endogenous nitric oxide (NO) synthase antagonist, asymmetric dimethylarginine (ADMA), a predictor of adverse outcome in patients with coronary artery disease (CAD) (Schnabel et al. 2005; Meinitzer et al. 2007; Wang et al. 2009; Lu et al. 2011), end-stage renal disease (Zoccali et al. 2001) and in the general population (Leong et al. 2008; Böger et al. 2009; Kiechl et al. 2009). Intriguingly, in contrast to a report of 2007 (Krzyzanowska et al. 2007), some analyses limited to T2DM subjects revealed no evidence of the detrimental ADMA prognostic effect (Lu et al. 2011; Anderssohn et al. 2014) or even a paradoxical association of adverse outcome with lower ADMA levels (Teerlink et al. 2006; Böger et al. 2009; Anderssohn et al. 2010). Additionally, ADMA levels were reportedly elevated (Abbasi et al. 2001; Konukoglu et al. 2008), similar (Böger et al. 2009) and even decreased (Päivä et al. 2003) in individuals with T2DM compared to controls without diabetes. This discrepancy was linked to a complex interplay of various factors (Teerlink et al. 2009; Anderssohn et al. 2010), including on the one side the hyperglycemia-induced inhibition of the major ADMA-degrading enzyme dimethylarginine dimethylaminohydrolase (DDAH) (Lin et al. 2002) and positive effects of insulin resistance by itself (Stühlinger et al. 2002; Marliss et al. 2006) on ADMA concentrations, and—on the other side—negative modulation of circulating ADMA by glomerular hyperfiltration at an initial stage of diabetic nephropathy (Päivä et al. 2003) and probably by the hyperinsulinemia-dependent potentiation of ADMA influx into the cells (Siroen et al. 2005; Eid et al. 2007). Moreover, relations between glucose metabolism and the L-arginine–NO pathway appear bidirectional because NO is able to influence insulin secretion, action and clearance (Kruszelnicka 2014).

Thus, our aim was to compare various biochemical markers of endothelial dysfunction and activation, including ADMA, in patients with stable CAD and T2DM stratified by metformin use.

Materials and methods

Patients

We studied 70 patients with established T2DM referred for elective coronary angiography owing to stable angina, receiving a standard CV medication and metformin or other oral antidiabetic drugs. The study subjects were being treated with low-dose aspirin, statins and angiotensin-converting enzyme inhibitors (ACEI) for $\geq$3 months prior to admission to limit the heterogeneity of the patients in terms of the use of drugs known to improve CV outcome in CAD, as described previously (Surdacki et al. 2010). The study population was divided into 2 groups according to metformin use for at least 1 year before index hospitalization. Patients who had been prescribed metformin combined with other oral antidiabetic agents had been a priori excluded. As patients on metformin constituted the vast majority of our CAD patients with T2DM, we recruited study subjects in the manner to ensure that the proportion of patients receiving metformin during preceding year would not be higher than 60 %.

Exclusion criteria included overt heart failure, congenital heart disease, significant valvular heart disease, arterial hypertension uncontrolled adequately by drugs, major surgery during past 6 months, any infections within preceding 2 months, estimated glomerular filtration rate (eGFR) below 45 mL/min per 1.73 m^2 of body surface area according to the Chronic Kidney Disease Epidemiology Collaboration formula, endocrine disorders other than diabetes, abnormal liver function, coexistent inflammatory or malignant diseases, relevant laboratory abnormalities in the

routine blood and urine analysis, and any chronic non-CV or non-diabetic medication (Surdacki et al. 2010).

In accordance with the Declaration of Helsinki, the ethics committee of our university approved the study and the patients gave informed written consent.

Blood sampling procedure

A portion of venous blood was drawn on admission from an antecubital vein after an overnight fast at the occasion of routine blood sampling for standard biochemical assays. In patients on metformin, blood sampling was performed prior to a temporary discontinuation of the drug before planned angiography. Blood for extended analyses was collected into plastic tubes containing ethylenediaminetetraacetic acid or no anticoagulant. After centrifugation ($4000\times g$; 4 °C, 10 min), plasma and serum were separated and stored at −80 °C until assayed.

Biochemical assays

Biochemical markers of endothelial dysfunction and activation included ADMA, monocyte chemotactic protein-1 (MCP-1) and soluble forms of vascular cell adhesion molecule-1 (sVCAM-1), intercellular adhesion molecule-1 (sICAM-1) and E-selectin (sE-selectin). Commercially available enzyme-linked immunosorbent assays (ELISAs) were used for the measurement of plasma levels of ADMA and its stereoisomer symmetric dimethylarginine (SDMA) (Immundiagnostik AG, Bernsheim, Germany), as well as plasma sVCAM-1, sICAM-1, MCP-1, and serum sE-selectin (R&D Systems, Minneapolis, MN, USA). The lower detection limit was 0.04 μM for ADMA and 0.05 μM for SDMA, and intra-assay and inter-assay coefficients of variation averaged 5.8 and 7.6 % (ADMA) and 4.8 and 7.0 % (SDMA), respectively. According to the manufacturer, cross-reactivity against L-arginine and other methylarginines was <0.02 % (SDMA or ADMA vs. L-arginine), <0.6 % (ADMA vs. SDMA), <0.5 % (SDMA vs. ADMA), and <0.5 % (SDMA vs. N^G-monomethyl-L-arginine). The lower detection limits for sVCAM-1, sICAM-1, MCP-1 and sE-selectin were 0.6 μg/L, 0.1 μg/L, 1.7 ng/L and 0.01 μg/L, respectively.

Statistical analysis

Data are presented as mean ± SD, medians (interquartile range) or *n* (%). The accordance with a normal distribution was checked using the Kolmogorov–Smirnov test. Patients on metformin were compared with the remainder by the unpaired two-sided Student's *t* test or Mann–Whitney *U* test for continuous data, and Fisher's exact test for proportions. Intergroup differences in biochemical indices of endothelial dysfunction and activation were also assessed by analysis of covariance (ANCOVA) to adjust for variables for which the intergroup *P* value or the *P* value in a univariate analysis was ≤0.15. ANCOVA results were shown as non-standardized regression coefficients (β, mean ± SEM); for continuous covariates β values reflect the change in a dependent variable for a given increase in the covariate, while for categorical predictors β value corresponds to the respective change in the subjects exposed to a factor of interest, i.e., metformin use, compared to a reference group without that exposure. Bivariate correlations were estimated by Pearson's or Spearman's correlation coefficients (*r*). A *P* value below 0.05 was inferred significant. Analyses were performed by means of the Statistica data analysis software system (version 10.0.1011.0; StatSoft Inc., Tulsa, OK, USA).

Results

Clinical characteristics were similar in patients stratified according to metformin use except for weak tendencies to higher body mass index and glycated hemoglobin in the study subjects receiving metformin (Table 1). Additionally, concomitant insulin therapy was insignificantly more frequent in the patients treated with metformin (Table 1). In 40 patients prescribed metformin, the mean daily dose of the drug was 1655 mg. Out of 30 patients receiving no metformin, 21 subjects (70 %) were treated with gliclazide modified release formulations in a mean dose of 59 mg daily.

Plasma levels of sVCAM-1 were significantly depressed and ADMA higher in the subjects on metformin (Table 2). The concentrations of sICAM-1 tended to be lower, whereas SDMA, sE-selectin and MCP-1 did not differ across the groups (Table 2).

Pooling all study subjects together, a weak negative correlation between ADMA and eGFR was found ($r = -0.19$, $P = 0.11$), whereas the strength of the respective relationship with renal function was moderate for SDMA ($r = -0.58$, $P < 0.001$). Plasma sVCAM-1 was associated with lower eGFR ($r = -0.31$, $P = 0.008$) and weakly with older age ($r = 0.25$, $P = 0.03$). Plasma sICAM-1 correlated to the concentrations of high-sensitivity C-reactive protein ($r = 0.29$, $P = 0.01$).

The associations of sVCAM-1 and ADMA with metformin use were maintained in ANCOVA upon inclusion of age, eGFR, body mass index and glycated hemoglobin as covariates ($P = 0.0015$ and $P = 0.025$ for sVCAM-1 and ADMA, respectively) (Table 3). Exclusion of insulin users from the analysis did not substantially change the results.

Neither ADMA nor SDMA correlated with sVCAM-1, sICAM-1, MCP-1 or sE-selectin ($P > 0.2$). None of these

Table 1 Patients' characteristics by metformin use

Characteristic	Metformin ($n = 40$)	No metformin ($n = 30$)	P value
Age (years)	67 ± 8	70 ± 10	0.3
Women, *n* (%)	16 (40)	13 (43)	0.8
Current smoking, *n* (%)	10 (25)	7 (23)	0.9
Body mass index (kg/m^2)	29.5 ± 4.0	27.9 ± 3.8	0.09
Waist circumference (cm)	105 ± 14	100 ± 17	0.2
Time from diabetes diagnosis (years)	6 (3–11)	5 (3–10)	0.7
HbA1c [%, (mmol/mol)]	7.3 ± 1.2 (56 ± 13)	6.9 ± 1.0 (52 ± 11)	0.14
Hypertension, *n* (%)	35 (88)	27 (90)	0.8
Multivessel CAD, *n* (%)	28 (70)	22 (73)	0.8
Estimated GFR (mL/min per 1.73 m^2)	75 ± 19	79 ± 22	0.4
LDL cholesterol (mM)	1.9 ± 0.8	2.0 ± 0.6	0.6
HDL cholesterol (mM)	1.1 ± 0.4	1.1 ± 0.3	0.7
Triglycerides (mM)	1.6 ± 0.6	1.4 ± 0.5	0.2
C-reactive protein (mg/L)	2.1 (1.0–3.4)	2.2 (1.1–3.6)	0.5
Hemoglobin (g/dL)	13.1 ± 1.7	12.8 ± 1.6	0.4
Drugs besides aspirin and statins, *n* (%)			
Insulin	14 (35)	6 (20)	0.17
ACEI	40 (100)	30 (100)	0.99
β-Blockers	38 (95)	27 (90)	0.4
Calcium channel blockers	13 (33)	8 (27)	0.6
Diuretics	20 (50)	13 (43)	0.6

Data are shown as mean ± SD, median (interquartile range) or *n* (%)

ACEI angiotensin-converting enzyme inhibitors, *CAD* coronary artery disease, *GFR* glomerular filtration rate, *HbA1c* glycated hemoglobin, *HDL* high-density lipoproteins, *LDL* low-density lipoproteins

Table 2 Biochemical indices of endothelial dysfunction and activation by metformin use

Characteristic	Metformin ($n = 40$)	No metformin ($n = 30$)	P value
ADMA (μM)	0.53 ± 0.09	0.48 ± 0.08	0.01
SDMA (μM)	0.49 ± 0.11	0.48 ± 0.09	0.7
sVCAM-1 (μg/L)	553 ± 148	668 ± 170	0.004
sICAM-1 (μg/L)	247 ± 62	275 ± 68	0.08
MCP-1 (ng/L)	217 (181–298)	220 (176–311)	0.6
sE-selectin (μg/L)	48 (30–79)	52 (31–84)	0.7

Data are shown as mean ± SD or median (interquartile range)

ADMA asymmetric dimethylarginine, *MCP* monocyte chemotactic protein-1, *SDMA* symmetric dimethylarginine, *sICAM-1* soluble intercellular adhesion molecule-1, *sVCAM-1* soluble vascular cell adhesion molecule-1

endothelial markers were related to body mass index, glycated hemoglobin, diabetes duration or insulin use ($P \geq 0.3$).

Discussion

Our principal finding is a differential association of metformin therapy with various biochemical markers of endothelial dysfunction and activation in patients with T2DM and CAD who exhibited lower sVCAM-1 and higher ADMA compared to their counterparts receiving other oral antidiabetic agents. Intriguingly, in our hands, the opposing directionality of intergroup differences was observed for the biomarkers, both of which had previously been positively linked to endothelial dysfunction and adverse CV outcome in CAD (Blankenberg et al. 2001; Schnabel et al. 2005; Meinitzer et al. 2007; Lu et al. 2011). Thus, discordant changes in sVCAM-1 and ADMA were rather unexpected. In addition, demographic and angiographic characteristics, glycemic control, lipids, renal function and co-administered CV drugs were similar in our subjects stratified by metformin use, which can suggest mechanisms other than a distinct pattern of CV risk factors or CV medication as an explanation of the intergroup differences in sVCAM-1 and ADMA. Because eGFR affects especially SDMA (Kiechl et al. 2009; Meinitzer et al. 2011) but also ADMA (Meinitzer et al. 2011) and sVCAM-1 (Jager et al. 2000), which was observed in our study subjects too, comparable eGFR and SDMA across the groups allowed us to exclude the variability of renal function as an underlying cause of our findings. Admittedly, the percentage of insulin users was higher, albeit not significantly, in patients receiving metformin. However, keeping in mind the ability of insulin to lower ADMA levels

Table 3 Levels of ADMA and sVCAM-1 according to metformin use by ANCOVA

Predictor	ADMA (μM)		sVCAM-1 (μg/L)	
	$\beta \pm$ SEM	*P* value	$\beta \pm$ SEM	*P* value
Metformin use (yes vs. no)	0.026 ± 0.011	0.025	−69 ± 22	0.0015
Covariates				
Estimated GFR (per rise by 10 mL/min per 1.73 m^2)	−0.010 ± 0.006	0.12	−24 ± 12	0.04
HbA1c (per 1-% increase)	−0.011 ± 0.010	0.3	27 ± 19	0.16
Body mass index (per rise by 1 kg/m^2)	−0.001 ± 0.003	0.8	−1 ± 5	0.9
Age (per 10-year increment)	–	–	53 ± 27	0.05

β mean non-standardized regression coefficient, *SEM* standard error of the mean, other abbreviations as in Tables 1 and 2

in humans (Siroen et al. 2005; Eid et al. 2007), this effect would rather decrease, not increase ADMA concentrations, whereas ADMA was higher in the study subjects on metformin. Finally, the observed differences were maintained after limitation of the analysis to patients receiving only oral antidiabetic drugs.

Of note, an adjustment for accompanying changes in glucose levels appears necessary to investigate the effects of antidiabetic drugs by themselves on ADMA, cell adhesion molecules and endothelium-dependent vascular function. An improved glycemic control coincided with decreases in ADMA (Asagami et al. 2002) and sVCAM-1 (Abbasi et al. 2004) after 3–4 months of metformin added to diet or a sulfonylurea compound in T2DM, and with a fall of sVCAM-1 after 16 weeks of metformin in a randomized placebo-controlled study in impaired glucose tolerance (Caballero et al. 2004). In sharp contrast, similar levels of ADMA and sVCAM-1 were observed after 4-month treatment with metformin or the prandial insulin secretagogue repaglinide at comparable glycemic regulation in a randomized crossover study of patients with T2DM (Lund et al. 2008). However, forearm microvascular dilation in response to acetylcholine was either increased despite similar glycemia (Mather et al. 2001) or unchanged at better glycemic control (Natali et al. 2004) after 12 or 16 weeks of metformin, respectively, with the reference to T2DM subjects randomized to placebo. Interestingly, 6 months of metformin therapy, in addition to a basal-bolus insulin regimen, improved flow-mediated dilation of the brachial artery in individuals with uncomplicated type 1 diabetes mellitus despite no differences in glucose concentrations compared to placebo (Pitocco et al. 2013).

Moreover, small yet significant reductions in sVCAM-1 and von Willebrand factor (vWF) after 16 weeks of metformin versus placebo, as add-on therapy to insulin, could not be explained by metformin-associated changes in glycemic control, conventional risk factors or insulin dose in 353 subjects with T2DM (de Jager et al. 2005). These findings were recently confirmed after the extension of the follow-up period until about 4 years (de Jager et al. 2014). Thus, decreased sVCAM-1 levels in our patients receiving metformin could also reflect the well-recognized ability of metformin to lower sVCAM-1 (Abbasi et al. 2004; Caballero et al. 2004; de Jager et al. 2005, 2014), while the association of higher ADMA with metformin use was rather unanticipated.

Clinical implications

Admittedly, whether associations observed in a small cross-sectional study reflect longitudinal drug effects remains disputable. However, if a biomarker is considered a CV prognosticator, differences in circulating concentrations of this biomarker between patients treated with different antidiabetic agents are also expected to be concordant to some degree with recognized effects of these drugs on prognosis. With regard to sVCAM-1, in a subsample of 631 population-based Hoorn study participants (out of whom 76 % were free of prevalent atherosclerotic CV disease) stratified by glucose tolerance status and followed for a median of 11.3 years, endothelial dysfunction—estimated on the basis of mean SD scores from the levels of sVCAM-1 and vWF—explained about 34 % of the excessive CV mortality risk associated with T2DM (de Jager et al. 2006). As to ADMA in the Hoorn cohort, the combined incidence of fatal and non-fatal CV events in the highest ADMA quintile was surprisingly decreased in individuals with T2DM, being yet increased in those without diabetes over a 10-year follow-up (Teerlink et al. 2006). Accordingly, it does not seem implausible to assume that both depressed sVCAM-1 and elevated ADMA in our CAD patients with diabetes on metformin might result from a common underlying mechanism that is also associated with better CV outcome in T2DM on metformin.

In addition to the Hoorn study, the surprising ability of diabetes status to modify the association of ADMA with prognosis over a mean follow-up of about 11 years was observed also in the Framingham Offspring Study with a higher risk with increasing ADMA quartiles in 2948 participants without diabetes at baseline and a non-significant

opposite trend in 372 subjects with diabetes (Böger et al. 2009). Furthermore, in a single-centre study of 997 consecutive patients referred for coronary angiography, ADMA was positively linked to the severity of coronary atherosclerosis and the incidence of major adverse CV events during a median follow-up of 2.4 years only in 638 subjects free of diabetes (Lu et al. 2011). In keeping with these findings, ADMA was unrelated to either prevalent (about 36 %) or incident CV disease in 783 subjects from the Edinburgh Type 2 Diabetes Study followed for 4 years (Anderssohn et al. 2014). On the other hand, ADMA was reportedly associated with the prevalence of macrovascular disease (about 50 %) and predicted future major adverse CV events in 125 patients with T2DM over a 2-year follow-up (Krzyzanowska et al. 2007). Additionally, ADMA was related to poor CV outcome at 2 years but not CAD extent in 162 men with T2DM undergoing coronary angiography exhibiting obstructive CAD in 82 % (Cavusoglu et al. 2010).

These inconsistent results could be partially attributable to differences in patients' characteristics and antidiabetic medication. The glycemic control can also interfere with ADMA prognostic ability because in a recent study of 270 subjects with T2DM (concomitant CAD in 78 %) ADMA predicted major CV events at about 6 years exclusively only in those with serum glycated hemoglobin over 6.5 % (48 mmol/mol) (Hsu et al. 2014). However, although a considerable proportion of patients included in these studies was presumably treated with metformin (Teerlink et al. 2006; Krzyzanowska et al. 2007; Böger et al. 2009; Cavusoglu et al. 2010; Lu et al. 2011; Anderssohn et al. 2014; Hsu et al. 2014), comparisons of ADMA levels according to metformin use have been made only in the last report (Hsu et al. 2014) with lower ADMA in patients on metformin. Nevertheless, this observation may be not applicable to other populations because those subjects with T2DM were of Asian origin in whom a defect of insulin secretion appears relatively more relevant compared to insulin resistance that prevails in Caucasian subjects with T2DM (Koshizaka et al. 2012).

It is to be pointed out that our cross-sectional findings do not allow for conclusions in terms of the risk of adverse CV events, nonetheless, some parallelism between the present small-scale report and discordant prognostic effects of sVCAM-1 and ADMA in large prospective studies in T2DM (de Jager et al. 2006; Teerlink et al. 2006; Böger et al. 2009) requires further investigation.

Mechanistic considerations

Irrespective of clinical investigations, mechanistic studies are indispensable to identify a mechanism of the interaction of ADMA prognostic ability with diabetes. This interaction has been linked to uncoupling of L-arginine oxidation from oxygen reduction by endothelial NO synthase (eNOS) (Böger et al. 2009; Anderssohn et al. 2010) brought about by the oxidation of the eNOS cofactor tetrahydrobiopterin in endothelial cells exposed to hyperglycemia (Crabtree et al. 2008), consistent with the notion of the endothelium being converted from a net superoxide scavenger by eNOS-derived NO into a net superoxide generator by uncoupled eNOS in T2DM (Hink et al. 2001; Guzik et al. 2002; Dixon et al. 2005). According to this concept (Anderssohn et al. 2010), ADMA would act as a "recoupling agent" counteracting eNOS uncoupling in uncomplicated diabetes, whereas recognized detrimental ADMA effects could prevail in advanced stages of diabetes accompanied by progressive vascular damage. Nevertheless, the lack of association of ADMA with adverse CV outcome in diabetes was reported not only in population-based cohorts including 783 T2DM subjects from the Edinburgh Type 2 Diabetes Study (de Jager et al. 2006; Teerlink et al. 2006; Böger et al. 2009; Anderssohn et al. 2014) with the prevalence of CV disease of 10–36 % at baseline, but also in those undergoing coronary angiography (Lu et al. 2011), out of whom two-thirds had obstructive CAD.

In addition, it was proposed that a structural similarity between ADMA and metformin may underlie their opposing biological effects due to a possible competition of these compounds at the level of transmembrane transport (Bestermann 2011). Following this hypothesis, higher ADMA levels in our T2DM patients on metformin could be related to impaired ADMA uptake into the cells with consequently lower intracellular ADMA, which might also offer an alternative explanation for the paradoxical association of increased circulating ADMA concentrations with better outcome in diabetes. Of note, metformin pretreatment attenuated liver damage, reduced hepatic ADMA content but increased plasma ADMA in experimental inflammatory liver injury, which can indicate the importance of an asymmetrical distribution of ADMA between the tissue and plasma (Bal et al. 2014). However, although ADMA at equimolar concentrations reduced the uptake of radiolabeled metformin by *Xenopus laevis* oocytes (Detaille et al. 2002), even a threefold excess of metformin did not affect cellular uptake of L-arginine by the cationic amino acid transporter-1, known to transport also ADMA, in human embryonic kidney cells (Strobel et al. 2012).

Interestingly, metformin and ADMA are common substrates of the organic cation transporter-2 and the multidrug and toxin extrusion protein-1 that are localized to the basal and luminal membrane of renal tubular cells, respectively, and mediate the uptake of small organic cations from the blood and their subsequent export into the urine (Strobel et al. 2013). Finally, in the renal cortex of Wistar–Kyoto rats, metformin upregulated the expression of the ADMA-synthesizing enzyme type I protein arginine methyltransferase without effects on the major ADMA-decomposing

enzyme DDAH (Tsai et al. 2014), thus suggesting a relevance of augmented ADMA synthesis (Chobanyan-Jürgens et al. 2011). Nevertheless, the significance of these potential interactions between metformin and ADMA metabolism has not been confirmed so far.

Strengths and weaknesses of the study

First, low number of our patients is the main limitation of the study. Additionally, a cross-sectional study design also constrains conclusions based on our findings and a randomized crossover study would be much more appropriate for this purpose. Nevertheless, our patients represented high-risk subjects with T2DM and CAD who exhibited similar clinical characteristics and were receiving a relatively uniform CV medication. Finally, the associations of ADMA and sVCAM-1 levels with metformin therapy were maintained in ANCOVA.

Second, we measured dimethylarginines by means of ELISA, whereas mass spectrometry-based chromatographic methods are the golden standard method for this purpose (Martens-Lobenhoffer et al. 2009). However, that we found intergroup differences in ADMA but not SDMA strengthens our findings. Third, because we studied subjects of Caucasian ancestry, our results and their hypothetical interpretation may be not applicable to other populations. Finally, the type of antidiabetic medication was determined during history taking and an objective verification with medical records was possible only in a minority of study subjects.

Conclusions

Metformin use appears differentially associated with sVCAM-1 and ADMA in patients with T2DM and stable CAD. Whether this observation might reflect different prognostic ability of these two endothelial markers for CV outcome in diabetes remains to be studied.

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Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in this study were in accordance with the ethical standards of the institutional ethics committee and with the 1964 Helsinki declaration and its later amendments.

Informed consent Informed consent was obtained from all individual participants included in the study.

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