Higher Plasma Methylglyoxal Levels Are Associated With Incident Cardiovascular Disease and Mortality in Individuals With Type 2 Diabetes

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OBJECTIVE

Methylglyoxal (MGO) is a reactive dicarbonyl compound and a potential key player in diabetic cardiovascular disease (CVD). Whether plasma MGO levels are associated with CVD in type 2 diabetes is unknown.

RESEARCH DESIGN AND METHODS

We included 1,003 individuals (mean ± SD age 59.1 ± 10.5 years, 69.3% male, and 61.6% with prior CVD) with type 2 diabetes from the Second Manifestations of ARTerial disease cohort (SMART). We measured plasma MGO levels and two other dicarbonyls (glyoxal [GO] and 3-deoxyglucosone [3-DG]) at baseline with mass spectrometry. Median follow-up of CVD events was 8.6 years. Data were analyzed with Cox regression with adjustment for sex, age, smoking, systolic blood pressure, total cholesterol, HbA_{1c}, BMI, prior CVD, and medication use. Hazard ratios are expressed per SD Ln-transformed dicarbonyl.

RESULTS

A total of 287 individuals suffered from at least one CVD event (*n* = 194 fatal events, *n* = 146 myocardial infarctions, and *n* = 72 strokes); 346 individuals died, and 60 individuals underwent an amputation. Higher MGO levels were associated with total (hazard ratio 1.26 [95% CI 1.11–1.42]) and fatal (1.49 [1.30–1.71]) CVD and with all-cause mortality (1.25 [1.11–1.40]), myocardial infarction (1.22 [1.02–1.45]), and amputations (1.36 [1.05–1.76]). MGO levels were not apparently associated with stroke (1.03 [0.79–1.35]). Higher GO levels were significantly associated with fatal CVD (1.17 [1.00–1.37]) but not with other outcomes. 3-DG was not significantly associated with any of the outcomes.

CONCLUSIONS

Plasma MGO and GO levels are associated with cardiovascular mortality in individuals with type 2 diabetes. Influencing dicaronyl levels may therefore be a target to reduce CVD in type 2 diabetes.

Type 2 diabetes remains a leading risk factor for the development of cardiovascular disease (CVD), and CVD mortality remains higher for individuals with diabetes than for those without diabetes (1). Therefore, disentangling the risk factors for developing CVD in people with diabetes remains a top priority. A key mediator in the association

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between diabetes and CVD may be the formation of dicarbonyl compounds, reactive glucose metabolites that interact with protein residues to form advanced glycation end products (AGEs) (2). Methylglyoxal (MGO), glyoxal (GO), and 3-deoxyglucosone (3-DG) have been identified as major dicarbonyl compounds (3). Of these, MGO has been identified as the most reactive dicarbonyl and is a potential key player in the development of diabetes complications (4,5). MGO may contribute to diabetic CVD through several mechanisms, including the development of chronic kidney disease (CKD) and low-grade inflammation (LGI) (6). In atherosclerotic plaques, MGO induces growth of the necrotic core, predisposing plaques toward rupture (7). Importantly, MGO can be detoxified by the glyoxalase system, and an emerging body of evidence links expression of its rate-limiting enzyme glyoxalase 1 to development of diabetes complications and CVD (8). We found that glyoxalase 1 is lower in ruptured plaques, and others found glyoxalase 1 to be a key gene in the development of coronary artery disease (7,9). Higher levels of glyoxalase 1 expression have also been linked to protection against CKD in humans with long-standing diabetes (10). These findings may suggest a causal role of MGO in diabetes complications. Indeed, we recently found that higher levels of MGO are associated with incident CVD in individuals with type 1 diabetes (11). However, it is unclear whether these findings also apply to type 2 diabetes. Type 2 diabetes is often accompanied by a more complicated cardiovascular risk profile than type 1 diabetes, and not only high glucose levels but also LGI and lipid peroxidation are major drivers of MGO levels (4,12). Whether and to what degree plasma levels of MGO are associated with CVD in individuals with type 2 diabetes are unknown, and the association between plasma MGO levels and CVD in people with diabetes should be further explored.

In addition to CVD, MGO has been identified as a major driver of diabetic neuropathy (13). Amputation of the lower limb, owing to ulceration or critical ischemia, is a severe complication of diabetes and is often the result of a combination of diabetic neuropathy and a high burden of atherosclerotic disease (14). Since MGO seems to be a driver of both these processes, high plasma MGO levels may foreshadow a high risk of lower-limb amputation, but this association to our knowledge has yet to be explored.

Therefore, we investigated, in a large cohort of individuals with type 2 diabetes, whether higher plasma levels of MGO, as well as the other major dicarbonyls GO and 3-DG, are associated with incident cardiovascular morbidity and mortality and lower-limb amputation. We also investigated whether these associations were explained by potential mediators by adjusting for markers of CKD and LGI.

RESEARCH DESIGN AND METHODS

Study Populations

The Secondary Manifestations of ARTerial disease (SMART) study is an ongoing prospective single-center cohort study at the University Medical Centre Utrecht. Study participants were newly referred to the hospital either with manifest atherosclerotic disease or for the management of cardiovascular risk factors (i.e., hypertension, hyperlipidemia, or diabetes) and invited by the outpatient physician to participate in the study. Patients were screened noninvasively for manifestations of atherosclerotic diseases and risk factors other than the qualifying diagnosis. Exclusion criteria were age $<$18 years, malignancy, dependency in daily activities, and insufficient fluency in the Dutch language. For the current study, we used a subsample of 1,010 SMART patients with type 2 diabetes enrolled between January 1996 and March 2006 to ensure sufficient follow-up times for adequate statistical power of the study. Diabetes was defined as a referral diagnosis of type 2 diabetes, self-reported type 2 diabetes, the use of glucose-lowering agents, or a fasting plasma glucose concentration of $\geq$7.0 mmol/L at baseline with initiation of glucose-lowering treatment within 1 year after inclusion. Patients for whom the dicarbonyl levels could not be assayed (n = 7) were excluded, resulting in 1,003 patients included for analyses.

Dicarbonyl Levels and Other Measurements

Plasma levels of MGO, GO, and 3-DG were measured with ultra-performance tandem mass spectrometry (UPLC-MS/MS) (15). Plasma (25 μL) was mixed with 75 μL d_8-*O*-phenylenediamine (10 mg d_8-*O*-phenylenediamine in 10 mL of 1.6 mol/L perchloric acid) in an Eppendorf cup. After an overnight (20 h) reaction at room temperature, shielded from light, 10 μL internal standard solution was added. Samples were mixed and subsequently centrifuged for 20 min at 21,000*g* at a temperature of 4°C; 10 μL was injected for UPLC-MS/MS analysis. Plasma samples were stored at −80°C until analysis. The interassay variations for MGO, GO, and 3-DG were 4.3, 5.1, and 2.2%, respectively (15). The techniques used for the laboratory tests have previously been described (16,17). In short, plasma total cholesterol, triglycerides, glucose, and creatinine were measured using commercial enzymatic dry chemistry kits (Johnson & Johnson). HDL cholesterol in plasma was determined using a commercial enzymatic kit (Boehringer Mannheim) after precipitation of LDL and VLDL with sodium phosphotungstate magnesium chloride. LDL cholesterol was calculated using the Friedewald formula. A urine sample was collected to measure albuminuria and creatinine excretion. Creatinine was measured using a commercial enzymatic dry chemistry kit (Johnson & Johnson), and microalbuminuria was determined with immunoturbidimetric assays (Boehringer Mannheim). Microalbuminuria was calculated as the ratio of albumin to creatinine (milligrams of albumin per millimoles of creatinine) and classified as normoalbuminuria (albumin-to-creatinine ratio [ACR] of $<$3), microalbuminuria (ACR $<$30), and macroalbuminuria (ACR $>$30). Serum hsCRP was measured by immunonephelometry (BN II System Nephelometer analyzer; Dade Behring, Eschborn, Germany).

Follow-up and Clinical End Points

SMART participants were biannually asked to complete a questionnaire on hospitalizations and outpatient clinic visits. When a possible event was reported, hospital discharge letters and results of relevant laboratory and radiology examinations were collected. Based on this information, all events were audited by three members of the SMART Study End Point Committee, comprising physicians from different departments. The primary end point of this study was incident CVD, a composite end point of incident nonfatal myocardial infarction, nonfatal stroke, and CVD mortality. Secondary end points were total and CVD-related mortality. In addition, we investigated associations with incident myocardial infarction, stroke, and amputation as separate end points. We refer to Supplementary Table 1 for definitions

of end points used in this study. Follow-up duration was defined as the period between study inclusion and first cardiovascular event or death from any cause, date of loss to follow-up, or the preselected date of 1 March 2015. For the main outcome, of the 1,003 participants, 108 (10.3%) were recorded as lost to follow-up and included in analyses until censoring occurred.

Statistical Analyses

All analyses were performed with SPSS, version 20, for Windows (SPSS, Chicago, IL). The plasma dicarbonyls and C-reactive protein (CRP) showed a skewed distribution and were ln transformed prior to further analyses. We calculated a composite score by calculating an average of the MGO, GO, and 3-DG *z* scores.

Logistic regression models were used to investigate cross-sectional associations between plasma *z* scores of ln MGO, ln GO, ln 3-DG, and the dicaronyl composite score and presence of albuminuria and estimated glomerular filtration rate (eGFR) $<$60 and 45 mL/min/1.73 m^2. Only 18 individuals had an eGFR $<$30 mL/min/1.73 m^2, so we did not analyze this category of CKD separately.

Cox proportional hazards regression models were applied to investigate associations between plasma *z* scores of ln MGO, ln GO, ln 3-DG, and the dicaronyl composite score and study end points with adjustment for sex and age (model 1). Further adjustments were performed for the major cardiovascular risk factors and glycemic control: BMI, systolic blood pressure, total cholesterol, current smoking, lipid-modifying and blood pressure–and glucose-lowering treatment, HbA_{1c}, and prior CVD (model 2).

Further analyses with adjustment for markers of CKD (adjustment for eGFR [model 3], presence of albuminuria [model 4], or both [model 5]) and CRP as a marker of LGI (model 6) were performed to evaluate the extent to which these pathophysiological processes could explain the associations between dicarbonyls and study outcomes. We did not observe any obvious nonlinearity of plasma dicarbonyl levels with any of the outcomes when we plotted hazard rates across tertiles of dicarbonyl levels over time. In additional analyses, we stratified our main analyses for sex and investigated whether any of the associations differed between men and women (sex $\times$ MGO, GO, or 3-DG) or presence of prior CVD (prior CVD $\times$ MGO, GO, or 3-DG) by adding interaction terms to model 2. We performed a fivefold multiple imputation to replace missing values (Supplementary Table 2). Results are reported for the pooled estimates.

RESULTS

Table 1 shows the baseline characteristics of the subpopulation of the SMART study that is the focus of this report. In Supplementary Tables 3–5, we report the baseline characteristics across tertiles of MGO, GO, and 3-DG. After a median follow-up of 8.6 years (minimum 0.1 years and maximum 18.3 years), 287 people developed incident CVD, while 346 participants died, of whom 194 died of CVD. In total, 146 and 72 participants developed incident myocardial infarction and stroke, respectively, and 60 underwent a lower-limb amputation.

Associations of Plasma MGO, GO, and 3-DG Levels With Markers of CKD

Higher plasma MGO and GO levels were associated with a higher prevalence of albuminuria (Table 2 [models 1 and 2]). This association was attenuated after adjustment for eGFR but remained statistically significant. Furthermore, higher plasma levels of MGO and GO were associated with a higher odds of having an eGFR $<$60 and 45 mL/min/1.73 m^2, independently of major risk factors for CVD and/or CKD and medication use (Table 2 [models 1 and 2]). Although higher 3-DG levels were consistently associated with a higher odds of having albuminuria or an eGFR $<$60 or 45 mL/min/1.73 m^2, this was not statistically significant after additional adjustment (Table 2 [model 2]).

Associations of MGO With Incident Cardiovascular Morbidity and Mortality

After adjustment for sex and age, higher plasma MGO levels were associated with incident CVD, all-cause and CVD mortality (Table 3 [model 1]), myocardial infarction, and incident amputation but not incident stroke (Table 4 [model 1]). Further adjustment for cardiovascular risk factors, HbA_{1c}, and medication use did not change the point estimates of these associations to an important extent (Tables 3 and 4 [model 2]). The associations between plasma MGO levels and total incident CVD, all-cause and CVD mortality (Table 3 [models 2–5]), and myocardial infarction (Table 4 [models 2–5]) were attenuated after adjustment for markers of CKD, in particular after adjustment for eGFR (Tables 3 and 4 [model 3]). Overall, the associations were not attenuated after adjustment for CRP as a marker of LGI (Tables 3 and 4 [model 2 vs. 6]). In contrast, adjustment of the association between higher plasma MGO levels and incident amputation for markers of CKD or CRP did not materially attenuate the association (Table 4 [models 2–6]).

Associations of GO With Incident Cardiovascular Morbidity and Mortality

After adjustment for sex and age, higher plasma GO levels were associated with CVD mortality but not with all-cause mortality or total CVD (Table 3 [model 1]). We found no associations between plasma GO levels and incident amputation or stroke, while we observed a trend for incident myocardial infarction (Table 4 [model 1]). Further adjustment for cardiovascular risk factors, HbA_{1c}, and medication use did not materially change the point estimates of these associations (Tables 3 and 4 [model 2]). The association between plasma GO levels and CVD mortality was strongly attenuated after adjustment for markers of CKD (Table 4 [models 2–5]), in particular, eGFR (Table 3 [model 3]). Overall, the associations were not materially attenuated by adjustment for CRP as a marker of LGI (Tables 3 and 4 [model 2 vs. 6]).

Associations of 3-DG With Incident Cardiovascular Morbidity and Mortality

We found no significant associations between plasma 3-DG levels and total CVD, CVD mortality, or all-cause mortality (Table 3 [models 1–6]). We found a significant association between plasma 3-DG levels and incident amputation (Table 4 [model 1]), which was attenuated after additional adjustment for cardiovascular risk factors, medication use, and HbA_{1c} and lost its statistical significance (Table 4 [model 2]). Further adjustments for either markers of CKD or CRP as a marker of LGI did not change the association (Table 4 [models 2–6]). We observed a trend toward an association between higher 3-DG levels and incident myocardial infarction and stroke (Table 4 [model 2]). Further adjustment for CKD attenuated the association between plasma 3-DG levels and incident myocardial

Table 1—Baseline characteristics according to total incident CVD at end of follow-up

	No incident CVD	Total incident CVD	Total SMART subset
n	716	287	1,003
Age (years)	57.4 ± 10.5	63.3 ± 9.3	59.1 ± 10.5
Sex (male)	66.1	77.4	69.3
HbA_{1c} (%)	7.4 ± 1.4	7.4 ± 1.4	7.4 ± 1.4
HbA_{1c} (mmol/mol)	57.0 ± 15.0	57.0 ± 15.0	57.0 ± 15.0
Glucose-lowering treatment	94.2	95.5	94.5
BMI (kg/m^2)	29.1 ± 5.2	28.1 ± 4.3	28.8 ± 5.0
Total cholesterol (mmol/L)	5.2 ± 1.5	5.2 ± 1.1	5.2 ± 1.4
HDL cholesterol (mmol/L)	1.2 ± 0.4	1.1 ± 0.3	1.1 ± 0.3
LDL cholesterol (mmol/L)	3.0 ± 1.1	3.2 ± 0.9	3.0 ± 1.0
Triglycerides (mmol/L)	1.9 (1.3–2.8)	1.9 (1.3–2.6)	1.9 (1.3–2.7)
Lipid-modifying treatment	45.5	48.9	45.9
Serum creatinine (μmol/L)	86.3 ± 26.3	108.7 ± 85.6	92.7 ± 51.8
eGFR (mL/min/1.73 m^2)	82.4 ± 21.5	72.5 ± 23.1	79.5 ± 22.4
Albuminuria (normo/micro/macro)	76.0/20.9/3.1	67.6/23.7/8.7	73.6/21.7/4.7
Systolic blood pressure (mmHg)	145.5 ± 20.2	149.3 ± 23.7	146.6 ± 21.3
Diastolic blood pressure (mmHg)	83.8 ± 11.0	81.1 ± 12.2	83.0 ± 11.4
Antihypertensive treatment	55.7	53.0	54.9
Smoking (yes)	26.7	33.0	28.7
CRP (mg/L)	2.5 (1.3–5.2)	3.7 (1.5–7.8)	2.8 (1.3–6.0)
MGO (nmol/L)	312.7 (279.5–362.5)	328.2 (286.2–391.5)	317.0 (282.1–368.2)
GO (nmol/L)	735.1 (585.8–883.2)	725.8 (587.4–923.5)	731.4 (586.7–890.8)
3-DG (nmol/L)	1,369.6 (1,139.1–1,716.3)	1,399.2 (1,140.7–1,839.6)	1,376.6 (1,140.7–1,748.7)
Prior CVD	56.8	81.1	61.6

Data are presented as means ± SD, median (interquartile range), or percentage, as appropriate, unless otherwise indicated. normo/micro/macro, normoalbuminuria/microalbuminuria/macroalbuminuria.

infarction (Table 4 [models 2–5]) but not the association between plasma 3-DG levels and incident stroke (Table 4 [models 2 and 3]). Further adjustment for CRP did not change the association between plasma 3-DG levels and stroke or myocardial infarction (Table 3 [model 2 vs. 6]).

Additional Analyses

For model 2 we additionally adjusted for triglycerides and HDL and LDL cholesterol levels to further explore whether lipid levels influenced the associations between plasma dicarbonyls and cardiovascular morbidity and mortality. This was not the case, as point estimates remained virtually the same after this additional adjustment for all analyses (data not shown). Similarly, additional adjustment for alcohol use did not overall change any of the associations (data not shown). Adjustment for inhibitors of the renin-angiotensin

Table 2—Multivariable-adjusted odds ratios (95% CI) for the associations among plasma dicarbonyls, albuminuria, and decreased eGFR

	M	Albuminuria (*n* = 265)	Macroalbuminuria (*n* = 47)	eGFR <60 mL/min/1.73 m^2 (*n* = 173)	eGFR <45 mL/min/1.73 m^2 (*n* = 64)
Ln MGO	1	1.52 (1.31–1.76)	2.05 (1.60–2.62)	2.56 (2.08–3.14)	5.41 (3.55–8.24)
	2	1.48 (1.26–1.72)	2.05 (1.58–2.66)	2.77 (2.21–3.47)	6.10 (4.08–9.12)
	3	1.29 (1.08–1.53)	1.36 (0.99–1.87)	—	—
Ln GO	1	1.37 (1.17–1.60)	1.72 (1.25–2.38)	1.53 (1.26–1.86)	2.70 (1.98–3.68)
	2	1.31 (1.00–1.54)	1.62 (1.13–2.32)	1.59 (1.29–1.96)	3.11 (2.19–4.41)
	3	1.21 (1.02–1.43)	1.18 (0.82–1.69)	—	—
Ln 3-DG	1	1.25 (1.08–1.44)	1.35 (1.01–1.81)	0.92 (0.77–1.09)	1.10 (0.84–1.42)
	2	1.10 (0.92–1.31)	1.21 (0.84–1.74)	0.97 (0.78–1.19)	1.25 (0.91–1.73)
	3	1.12 (0.94–1.34)	1.22 (0.84–1.79)	—	—
Dicarbonyl score	1	1.48 (1.24–1.76)	1.97 (1.48–2.62)	1.62 (1.36–1.94)	2.83 (2.15–3.74)
	2	1.39 (1.07–1.65)	2.00 (1.42–2.82)	1.87 (1.52–2.30)	4.01 (2.81–5.73)
	3	1.27 (1.07–1.51)	1.33 (0.93–1.92)	—	—

Data were analyzed using logistic regression analyses. Odds ratio is expressed per SD increase of Ln-transformed plasma dicarbonyl. Model 1, adjusted for age and sex; model 2, model 1 adjustments plus adjustment for total cholesterol, BMI, current smoking and systolic blood pressure, prior CVD, HbA_{1c}, and lipid-modifying and blood pressure– and glucose-lowering treatment; and model 3, model 2 adjustments plus adjustment for eGFR. M, model.

Table 3—Multivariable-adjusted hazard ratios (95% CI) for the associations among plasma dicarbonyls, incident CVD, and mortality

	M	Total CVD (*n* = 287)	CVD mortality (*n* = 194)	All-cause mortality (*n* = 346)
Ln MGO	1	1.25 (1.11–1.40)	1.43 (1.25–1.64)	1.27 (1.14–1.41)
	2	1.26 (1.11–1.42)	1.49 (1.29–1.71)	1.25 (1.11–1.40)
	3	1.14 (1.00–1.31)	1.31 (1.11–1.54)	1.13 (1.00–1.28)
	4	1.22 (1.07–1.38)	1.40 (1.21–1.62)	1.19 (1.06–1.33)
	5	1.12 (0.98–1.28)	1.26 (1.07–1.49)	1.10 (0.97–1.24)
	6	1.24 (1.09–1.40)	1.45 (1.26–1.67)	1.22 (1.09–1.37)
Ln GO	1	1.06 (0.95–1.20)	1.17 (1.01–1.35)	1.07 (0.96–1.19)
	2	1.06 (0.93–1.20)	1.17 (1.00–1.37)	1.02 (0.92–1.14)
	3	1.01 (0.89–1.14)	1.09 (0.93–1.27)	0.98 (0.88–1.09)
	4	1.03 (0.91–1.17)	1.13 (0.96–1.33)	0.99 (0.89–1.11)
	5	0.99 (0.88–1.12)	1.06 (0.90–1.24)	0.97 (0.86–1.07)
	6	1.06 (0.93–1.20)	1.17 (1.00–1.37)	1.03 (0.92–1.15)
Ln 3-DG	1	1.10 (0.97–1.23)	1.11 (0.96–1.28)	1.05 (0.94–1.17)
	2	1.07 (0.93–1.24)	1.05 (0.87–1.27)	0.93 (0.81–1.06)
	3	1.09 (0.95–1.26)	1.08 (0.90–1.30)	0.95 (0.83–1.08)
	4	1.06 (0.92–1.23)	1.03 (0.86–1.25)	0.91 (0.79–1.04)
	5	1.08 (0.94–1.25)	1.06 (0.88–1.27)	0.92 (0.81–1.06)
	6	1.07 (0.92–1.25)	1.05 (0.87–1.27)	0.93 (0.81–1.06)
Dicarbonyl score	1	1.16 (1.03–1.31)	1.29 (1.12–1.49)	1.15 (1.03–1.28)
	2	1.17 (1.02–1.34)	1.32 (1.11–1.57)	1.08 (0.95–1.22)
	3	1.10 (0.96–1.26)	1.20 (1.01–1.43)	1.02 (0.90–1.15)
	4	1.14 (0.99–1.31)	1.27 (1.07–1.51)	1.04 (0.91–1.18)
	5	1.08 (0.94–1.24)	1.17 (0.98–1.39)	0.98 (0.87–1.12)
	6	1.16 (1.01–1.33)	1.31 (1.10–1.56)	1.07 (0.95–1.22)

Data were analyzed using Cox regression analyses. Hazard ratio is expressed per SD increase of Ln-transformed plasma dicarbonyl. Model 1, adjusted for age and sex; model 2, model 1 adjustments plus adjustment for total cholesterol, BMI, current smoking and systolic blood pressure, prior CVD, HbA_{1c}, and lipid-modifying and blood pressure– and glucose-lowering treatment; model 3, model 2 adjustments plus adjustment for eGFR; model 4, model 2 adjustments plus adjustment for presence of albuminuria; model 5, model 2 adjustments plus adjustment for eGFR and presence of albuminuria; and model 6, model 2 adjustments plus adjustment for Ln CRP. M, model.

system instead of blood pressure–lowering medication in general also did not influence the results (data not shown).

We also combined plasma levels of MGO, GO, and 3-DG in a composite *z* score. Although a combined dicarbonyl score was overall associated with markers of CKD and incident CVD (Tables 2–4), these associations were not stronger than for plasma MGO levels alone.

We stratified our main analyses for sex, and we observed associations between plasma MGO, GO, and 3-DG levels and incident CVD, all-cause mortality, and CVD mortality that appeared stronger in women than in men (Supplementary Table 6 [model 2]). This was statistically significant at $P_{interaction} < 0.1$ for all-cause and CVD mortality for MGO and GO, but we found no significant interactions with sex for associations between plasma MGO, GO, and 3-DG levels and incident CVD, our primary outcome (Supplementary Table 7). Associations also did not differ between men and women for any of the plasma dicaronyl levels and incident myocardial infarction, stroke, and amputation ($P_{interaction} > 0.1$ [data not shown]). In addition, we found no interactions for prior CVD for associations between plasma MGO, GO, and 3-DG levels and any of the study outcomes (Supplementary Table 7). In addition, we found no interactions for prior CVD for associations between plasma MGO, GO, and 3-DG levels and any of the study outcomes (Supplementary Table 7). However, the number of events in participants without prior CVD was low, and although similar to results for the overall analyses, the associations were no longer statistically significant in participants without prior CVD after adjustment for sex and age when we stratified the analysis for prior CVD (Supplementary Table 8).

CONCLUSIONS

The main finding of this study was that plasma MGO levels are associated with incident CVD, all-cause and CVD mortality, myocardial infarction, and lower-limb amputation in individuals with type 2 diabetes. For the other dicarbonyls, we observed similar, but weaker, associations between GO levels and fatal CVD and no significant associations with the other CVD outcomes. We found no significant associations between 3-DG and any of the CVD outcomes, although we observed a trend for incident myocardial infarction, stroke, and lower-limb amputation.

This is the first study demonstrating an association between plasma MGO levels and incident CVD in type 2 diabetes. Our current findings are largely in line with our recent report on associations between plasma MGO, GO, and 3-DG levels and incident CVD in type 1 diabetes (11). A major difference from our previous study is that, in the current study, we found that adjustment for markers of CKD attenuated associations between MGO and GO and cardiovascular mortality as well as incident total CVD and ischemic heart disease. This observation suggests that dicarbonyl stress may lead to increased cardiovascular risk in type 2 diabetes in part through development of CKD. This is indeed in line with recent human work that implicates MGO in the development of CKD (10,18). A recent report has investigated associations between several dicarbonyl-derived AGEs and incident CVD in participants with type 2 diabetes (19), and the overall associations between these MGO-derived AGEs and incident CVD seemed to attenuate largely after adjustment for prior CVD and other confounders, while in our report associations between MGO and incident CVD remained largely independent of cardiovascular confounders, including prior CVD. These are interesting findings that suggest that plasma AGE levels may not directly be used as plasma biomarkers of their respective dicaronyl precursors. In line with the current paradigm that MGO is the most reactive dicarbonyl in vivo (5), we found that the strongest associations were between plasma MGO levels and cardiovascular mortality and morbidity in comparison with plasma GO and 3-DG levels. However, we observed trends between plasma GO and 3-DG levels and some of the

Table 4—Multivariable-adjusted hazard ratios (95% CI) for the associations among plasma dicarbonyls, incident myocardial infarction, stroke, and amputation

	M	Myocardial infarction (*n* = 146)	Stroke (*n* = 72)	Amputation (*n* = 60)
Ln MGO	1	1.22 (1.03–1.43)	1.06 (0.82–1.36)	1.42 (1.13–1.79)
	2	1.21 (1.01–1.45)	1.02 (0.80–1.31)	1.36 (1.05–1.76)
	3	1.15 (0.95–1.41)	0.95 (0.73–1.23)	1.41 (1.06–1.87)
	4	1.17 (0.98–1.41)	1.02 (0.79–1.31)	1.29 (1.00–1.66)
	5	1.13 (0.93–1.37)	0.95 (0.73–1.23)	1.36 (1.02–1.80)
	6	1.19 (1.00–1.43)	1.02 (0.80–1.31)	1.32 (1.03–1.70)
Ln GO	1	1.13 (0.95–1.35)	0.99 (0.78–1.24)	1.28 (0.97–1.67)
	2	1.14 (0.95–1.37)	0.97 (0.77–1.21)	1.17 (0.89–1.54)
	3	1.11 (0.92–1.33)	0.94 (0.75–1.17)	1.16 (0.88–1.54)
	4	1.12 (0.93–1.35)	0.96 (0.77–1.20)	1.13 (0.86–1.49)
	5	1.09 (0.91–1.32)	0.94 (0.75–1.18)	1.14 (0.86–1.51)
	6	1.14 (0.95–1.37)	0.97 (0.77–1.21)	1.15 (0.88–1.51)
Ln 3-DG	1	1.17 (0.99–1.38)	1.22 (0.97–1.53)	1.42 (1.10–1.84)
	2	1.18 (0.97–1.44)	1.26 (0.95–1.68)	1.24 (0.91–1.69)
	3	1.20 (0.98–1.46)	1.28 (0.96–1.70)	1.24 (0.91–1.70)
	4	1.18 (0.96–1.44)	1.26 (0.95–1.68)	1.23 (0.89–1.68)
	5	1.19 (0.97–1.45)	1.28 (0.96–1.70)	1.22 (0.89–1.68)
	6	1.18 (0.97–1.44)	1.26 (0.95–1.68)	1.25 (0.91–1.70)
Dicarbonyl score	1	1.22 (1.03–1.44)	1.09 (0.87–1.38)	1.49 (1.16–1.91)
	2	1.24 (1.02–1.51)	1.07 (0.83–1.38)	1.37 (1.02–1.83)
	3	1.20 (0.99–1.46)	1.03 (0.80–1.33)	1.37 (1.02–1.85)
	4	1.22 (1.00–1.48)	1.07 (0.82–1.38)	1.32 (0.99–1.77)
	5	1.19 (0.97–1.45)	1.03 (0.80–1.33)	1.35 (0.99–1.82)
	6	1.24 (1.02–1.50)	1.07 (0.83–1.38)	1.35 (1.01–1.80)

Data were analyzed using Cox regression analyses. Hazard ratio is expressed per SD increase of Ln-transformed plasma dicarbonyl. Model 1, adjusted for age and sex; model 2, model 1 adjustments plus adjustment for total cholesterol, BMI, current smoking and systolic blood pressure, prior CVD, HbA_{1c}, and lipid-modifying and blood pressure– and glucose-lowering treatment; model 3, model 2 adjustments plus adjustment for eGFR; model 4, model 2 adjustments plus adjustment for presence of albuminuria; model 5, model 2 adjustments plus adjustment for eGFR and presence of albuminuria; and model 6, model 2 adjustments plus adjustment for Ln CRP. M, model.

cardiovascular outcomes, and therefore we cannot rule out that these dicarbonyls may also play a role in the development of cardiovascular complications in diabetes. When we stratified our analyses according to sex, associations for all-cause and CVD mortality were stronger in women than in men. For total CVD, we observed a similar pattern, but this difference was not statistically significant. Furthermore, we observed no apparent difference between men and women for incident myocardial infarction, stroke, and amputations. We currently have no plausible biological explanation for these findings. We are also not aware of any differences in GLO1 activity between men and women, and levels of MGO, GO, and 3-DG were similar for men and women (data not shown). In line with this, we previously found no statistical interactions for sex when we studied dicarbonyls or their derived AGEs in prior studies in type 1 and type 2 diabetes (3,11,20,21). Therefore, these findings are interesting but should be interpreted very cautiously and as hypothesis generating only.

The finding that higher plasma dicarbonyl levels are associated with a higher rate of lower-limb amputations is intriguing, as diabetic foot ulcerations represent a severe and poorly understood complication of diabetes, for which few effective therapeutic strategies exist (14). Interestingly, adjustment for eGFR did not change the association between plasma MGO levels and amputations. Although we cannot fully rule out that this was due to chance, this observation indeed suggests that the association between MGO and amputations is mediated not strictly through atherosclerotic disease of the lower limbs but also via other disease mechanisms such as diabetic neuropathy (13), to which plasma MGO levels have been linked (22). Perhaps the inhibition of dicarbonyl stress may offer a therapeutic axis to prevent this severe diabetes complication. Surprisingly, we found no apparent associations between any of the dicarbonyls and incident stroke. This is in contrast with previous work where we found that the MGO-derived AGE N^{ε}-(carboxyethyl)lysine is associated with incident stroke (20) and that MGO-derived AGEs are higher in rupture-prone carotid plaques (7). Therefore, this finding may be due to lack of statistical power on this end point.

The current report allowed for adjustment of a large range of potential confounders, including HbA_{1c} and cardiovascular risk–modifying drugs. Therefore, the current study suggests that dicarbonyl stress is a residual risk factor for CVD in diabetes that warrants specific treatment. This may help close the persistent gap in cardiovascular mortality between individuals with and without diabetes (1). Several strategies may achieve lowering of dicarbonyl stress. Bariatric surgery achieves a rapid reduction in dicarbonyl stress in type 2 diabetes (23), and the question should be addressed of whether some of the beneficial effects of bariatric surgery on risk of CVD are explained by reduced dicarbonyl stress. Another approach under active investigation is enhanced detoxification of MGO through induction of GLO1 expression with a coformulation of resveratrol and hesperetin (24). Moreover, direct quenchers of MGO, such as by the vitamin B6 analog pyridoxamine, are under active investigation in rodents and in humans (25–27).

A major strength of our study is that we had a large sample of individuals with type 2 diabetes at high risk of cardiovascular complications with substantial follow-up. Furthermore, we used UPLC-MS/MS to precisely quantify MGO, GO, and 3-DG levels. However, our study also has several limitations. We studied individuals with diabetes who already have a high burden of cardiovascular disease. We therefore do not know whether the results from the current paper can be extrapolated to patients with newly diagnosed diabetes with a lower cardiovascular risk. In addition, at baseline not all participants met current blood pressure, lipid, and glycemic control targets for optimal risk management, and therefore we do not know whether our current results apply to a population with optimized cardiovascular risk management. Furthermore, in the current report we could only include CRP as a marker of LGI.

We therefore may have underestimated the mediating effect of LGI on the associations between dicarbonyls and CVD outcomes. Furthermore, because MGO production is increased in atherosclerotic plaques (7), our current study cannot fully rule out reversed causality (i.e., that an increased plaque burden increases plasma MGO levels, which in turn are associated with more cardiovascular events). However, we found no significant interaction by prior CVD in the associations between the plasma dicarbonyls and cardiovascular outcomes, and in our previous study with type 1 diabetes, atherosclerotic burden was low at baseline and we observed similar associations between plasma MGO levels and incident CVD (11). Additionally, we cannot exclude that we may have over- or underestimated certain associations due to limited events in certain subpopulations (participants without prior CVD) or with specific secondary end points (stroke, amputation)—both leading to a reduced statistical power.

In conclusion, the current study is the first to show the importance of plasma MGO levels in type 2 diabetes–associated CVD and mortality in humans. This study underlines the potential interest of identified inhibitors of MGO formation in efforts to curtail diabetes complications.

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