Methylglyoxal Is a Predictor in Type 2 Diabetic Patients of Intima-Media Thickening and Elevation of Blood Pressure

Susumu Ogawa, Keisuke Nakayama, Masaaki Nakayama, Takefumi Mori, Masato Matsushima, Masashi Okamura, Miho Senda, Kazuhiro Nako, Toshio Miyata, Sadayoshi Ito

Abstract—We test whether plasma level of methylglyoxal (MG) is an independent risk factor predicting the progression of diabetic macroangiopathy or microangiopathy in type 2 diabetic patients. We measured in 50 type 2 diabetic patients plasma levels of MG and 3-deoxyglucosone (DG) using an electrospray ionization-liquid chromatography-mass spectrometry. We assessed the correlations between baseline levels of MG or DG and the percentage changes after 5 years of clinical parameters linked to diabetic macroangiopathy or microangiopathy, that is, intima-media thickness (IMT), systolic blood pressure (SBP), the amount of urinary albumin excretion (ACR), pulse wave velocity (PWV), and estimated glomerular filtration rate (eGFR). Multiple regression analysis was performed using the percentage changes in IMT, SBP, ACR, PWV, and eGFR over the 5-year period as the independent or objective variables and the values of MG, DG, glycohemoglobin A1c, body mass index, triglyceride, and diabetic duration at the baseline as the dependent variables. The values of IMT, PWV, SBP, and ACR all increase, but eGFR reduces with time during the 5-year period. Baseline level of MG correlates significantly with the percentage changes of IMT, SBP, ACR, PWV, and eGFR, whereas that of DG does only with ACR. A multiple regression analysis reveals that MG is an independent risk factor for the percentage changes of IMT, PWV, and SBP but not for those of ACR and eGFR. DG is an independent risk factor for the percentage change of ACR. MG is a predictor in type 2 diabetic patients of intima-media thickening, of increase of PWV, and of elevation of SBP. (Hypertension. 2010;56:471-476.)

Key Words: methylglyoxal ■ 3-deoxyglucosone ■ diabetic macroangiopathy ■ hypertension ■ intima-media thickness ■ pulse wave velocity

Under hyperglycemia and/or oxidative stress in diabetes mellitus, a variety of toxic α-oxoaldehydes are produced, and these in turn react with protein amino groups, eventually leading to formation of advanced glycation end products (AGEs).1-3 These α-oxoaldehydes also interfere with various cellular functions, independent of their effect on AGE modification of proteins, and influence the intracellular signaling by multiple pathways.1-3

Among toxic α-oxoaldehydes, the present studies were focused on methylglyoxal (MG), because the in vitro studies and animal experiments in experimental diabetic models by us and others have suggested that MG is pathologically involved in the progression of both macroangiopathy and microangiopathy: MG plays a major role in vascular damage to endothelial cells and in the development of hypertension, of insulin resistance, and of nephropathy.1-10

The primary biosynthetic pathway of MG in diabetic patients remains elusive, but MG is known to be produced from a variety of sources. That is, MG can be produced not only from glucose but also from a variety of substances and is not necessarily produced from hyperglycemia only.1-2,11

Elevated blood concentrations of MG have been reported in type 2 diabetics,5-12 and it has been reported that plasma-free MG-derived hydroimidazolone was higher in the type 1 diabetics as compared with the nondiabetics.13 An 18-year follow-up study showed that high baseline serum levels of the MG-derived hydroimidazolone type of AGE-modified proteins were associated with cardiovascular disease mortality in nondiabetic women.14

Interestingly, postprandial hyperglycemia, a factor contributing to the development of macroangiopathy, dramatically increases the intracellular accumulation of MG,15-16 Furthermore, biguanide, an agent that effectively suppresses macroangiopathy independent of its blood glucose-lowering effect, significantly lowers the production of MG.17-19

Taken together, these findings strongly suggest the contribution of MG to diabetic angiopathy, especially macroangiopathy. Unfortunately, there have been no studies examining whether elevated plasma MG levels are an independent risk factor predicting the progression of diabetic macroangiopathy or microangiopathy.

Received May 20, 2010; first decision June 5, 2010; revision accepted June 21, 2010.
From the Division of Nephrology, Endocrinology, and Vascular Medicine (S.O., K.Naka., M.S., K.Nako, S.I.), Research Division of Dialysis and Chronic Kidney Disease (M.Naka.), Center for the Advancement of Higher Education (S.O.), and Center for Translational and Advanced Research (T.Mo., M.O., T.Mi.), Tohoku University, Sendai, Japan; Division of Clinical Epidemiology (M.M.), Jikei University School of Medicine, Tokyo, Japan.
Correspondence to Susumu Ogawa, Division of Nephrology, Endocrinology, and Vascular Medicine, Tohoku University Hospital, 1-1 Seiryo-machi, Aoba-ku, Sendai 980-8574, Japan. E-mail ogawa-s@mail.tains.tohoku.ac.jp
© 2010 American Heart Association, Inc.
Hypertension is available at http://hyper.ahajournals.org
DOI: 10.1161/HYPERTENSIONAHA.110.156786
In the present study, therefore, we measured the plasma levels of MG and 3-deoxyglucose (DG),20,21 another α-oxoaldehyde, in 50 type 2 diabetic patients using electrospray ionization-liquid chromatography-mass spectrometry5 and assessed the correlations between the baseline levels of MG or DG and the percentage of changes in the following clinical parameters linked to diabetic macroangiopathy or microangiopathy between baseline and a 5-year follow-up examination: intima-media thickness (IMT), pulse wave velocity (PWV), systolic blood pressure (BP), SBP, the amount of urinary albumin excretion (ACR), and estimated glomerular filtration rate (eGFR). It has been reported that IMT, PWV, SBP, ACR, and eGFR are predictive of future vascular events and that DG is a factor closely related to the advancement of microangiopathy.20 It is, thus, important to clarify the relationship between these parameters and MG.

This study was carried out to verify our hypothesis that elevated levels of MG and DG are predictive of the development or progression of diabetic angiopathy. We, therefore, examined the correlation between baseline values of MG and DG and diabetic renovascular complications at a 5-year follow-up in patients with type 2 diabetes mellitus. The present study provides the first clear evidence that MG is a predictor of intima-media thickening, vascular stiffening, and elevation of BP in type 2 diabetics.

Methods

This research is a 5-year prospective follow-up study, targeting 50 type 2 diabetes patients who visited our outpatient department during the 3 months from April to June 2003 and who gave their consent to participate in the study. From July to September 2003, we collected early morning initial urine samples from the subjects and collected blood samples in the early morning after fasting. The blood was immediately centrifuged after being drawn to collect the plasma and then immediately stored frozen at a temperature of −80°C. After the samples of all 50 of the subjects were collected, we measured MG and DG at the same time.

The following parameters were measured at baseline: height, body weight, BP, serum creatinine (Cre), triglycerides (TGs), total cholesterol, high-density lipoprotein cholesterol, glycohemoglobin A1c (HbA1c), high-sensitive C-reactive protein, atrial natriuretic peptide, cholesterol, high-density lipoprotein cholesterol, glycohemoglobin A1c (HbA1c), high-sensitive C-reactive protein, atrial natriuretic peptide, bile natriuretic peptide, adiponectin, tumor necrosis factor α-α (TNFα), monocyte chemotactic protein (MCP) 1, interferon-inducible protein 10, interleukin (IL) 6, IL-18, vessel endothelial growth factor, urinary pentosidine, urinary 8-epi-prostaglandin F2α, 8-hydroxydeoxyguanosine (8-OHdG), ACR, type IV collagen, IMT, PWV, ankle brachial index (ABI), and ocular fundus data. The plasma levels of MG and DG were measured simultaneously. We also measured the IMT, PWV, Cre, ACR, and SBP values and calculated eGFR, which was calculated by the following formula: eGFR = [194 Cre]⁻¹⁰⁹⁴ × age⁻⁰·²⁸⁷ [female × 0.739], at a follow-up examination performed 5 years after baseline, and examined the potential correlations between MG and DG and the 5-year changes in each of these 5 parameters. The present study was conducted after obtaining informed consent from all of the subjects, and the study protocol was approved by the ethics committees of Tohoku University Hospital.

Measurements

Quantification of MG and DG

MG and DG levels were assayed by derivatization with o-phenylenediamine and electrospray ionization-liquid chromatography-mass spectrometry of the resulting quinoxalines, as reported previously.4 Briefly, plasma samples were deproteinized with perchloric acid, and 2,3-dimethylquinoxaline as an internal standard and o-phenylenediamine as a derivatizing agent were added to the supernatant. The samples were incubated at 4°C for 2 hours and then applied to a prepared C18 SPE column and filtrated through 0.2-μm filters into sample vials. Derivatized MG and DG were analyzed by high-performance liquid chromatography (Agilent 1100 series, Agilent Technologies) and electrospray ionization-mass spectrometry using a time-of-flight mass spectrometer (Acquity TOF JMS-T100LC, JEOL). Derivatives were resolved by reverse-phase chromatography on a C18 Column (Cadenza CD-C18, 2.0×150 mm, Imtakt).

We modified the analytic conditions of liquid chromatography-mass spectrometry to obtain more precise data. The gradient speed of the mobile phase was slowed (from 6 to 10 minutes), and the mass/charge ratio (m/z) was detected more precisely (from m/z 145.00 to m/z 145.07 for MG and from m/z 235.00 to m/z 235.11 for DG). According to this modified method, the linear calibration curves relating peak area ratio with MG and DG concentrations were observed in the range of 50 to 3200 nmol/L (Figure S1, available in the online Data Supplement at http://hyper.ahajournals.org).

To evaluate interference in the assay of MG and DG in plasma samples, we compared a simple calibration curve with a standard addition curve prepared by adding increasing amounts of MG and DG to plasma samples. The slopes of the standard addition curves from plasma samples (Figure S2) were significantly different from the slopes of the simple calibration curves (Figure S1). However, the obtained slopes from 3 different healthy volunteers were very similar (Figure S2). Therefore, the average value of the slopes obtained from the standard addition curves with healthy control plasma was used to determine the MG and DG levels in plasma samples. According to this modified method, the mean plasma MG and DG levels in 10 healthy volunteers were 137±17 and 363±33 nmol/L, respectively.

The plasma MG levels derived using this modified method were lower than those we derived previously using the original previous data derived from the previous method,4 but significant correlations were observed between the previous and modified methods for 30 plasma samples: 10 from healthy controls and 20 from patients undergoing dialysis (P<0.0001; Figure S3). The raw data from subjects in this study were evaluated by the previous method, so in this article we presented the data converted with the regression equation, as shown in Figure S3. The intraday coefficients of variation for the assay of MG and DG with the modified method were 1.9% and 2.0% (n=5), and the interday coefficients of variation were 4.3% and 12.0% (n=5), respectively.

Quantification of Oxidative Stress Markers and Inflammatory Markers

Plasma adiponectin, MCP-1, interferon-inducible protein 10, IL-6, IL-18, TNFα, and vessel endothelial growth factor were measured via ELISA using, respectively, an Adiponectin ELISA kit (Ohtsuka Pharmaceutical Co. Ltd), an MCP-1 ELISA kit (R&D Systems), a human IP-10 ELISA kit (R&D Systems, Inc), an IL-6 ELISA kit (R&D Systems), a human IL-18 ELISA kit (Medical and Biological Laboratories), an Ultra Sensitivity TNFα ELISA kit (BioSource International), and a human vessel endothelial growth factor Immunoassay kit (R&D Systems Inc). Urinary 8-epi-prostaglandin F2α and 8-OHdG were measured using an 8-isoprostane enzyme immunoassay kit (Cayman Chemical Co) and ELISA kit (Japan Institute for the Control of Aging), respectively, whereas urinary pentosidine was measured using high-performance liquid chromatography. Both were corrected according to the level of urinary creatinine excretion.

Vascular Evaluation

The PWV and ABI were measured using Form PWV/ABI, version 112 (Colin Electronics Co, Ltd), and IMT, by the ATL Ultramark HDI 5000 Ultrasound System (Bothell).

Statistical Study

The data for patients whose actual measurement values (or log-converted values) showed normal distributions were recorded as the mean±SEM, and the data for patients who did not show a normal distribution were recorded as the median (range). The comparisons between the baseline and 5-year follow-up values of IMT, PWV,
SBP, ACR, and eGFR were carried out using the Student t test (for IMT, PWV, SBP, and eGFR) or the Wilcoxon signed-ranked test (for ACR). The correlation at baseline between MG and the various parameters was studied using single regression analysis, and the correlation between the percentage changes in IMT, PWV, SBP, ACR, and eGFR over the 5-year interval and either MG or DG was also investigated using single regression analysis. Finally, we performed a multiple regression analysis using the percentage changes in IMT, PWV, ACR, SBP, and eGFR over the 5-year period as the dependent or objective variables and the values of MG, DBP, HbA1c, TG, body mass index (BMI), diabetic duration, and SBP at the baseline as the independent variables. Correlations were determined by the Spearman rank correlation test, with P values of <0.05 regarded as significant.

**Results**

The baseline characteristics of the patients in this study, that is, the clinical information, the serum and urinary levels of the various parameters measured, and the main drugs administered, are shown in Table 1. At the initiation of the study, a weak but significant correlation was observed between each of MG and DG (r = 0.19; P < 0.05), BMI (r = 0.18; P < 0.05), SBP (r = 0.26; P < 0.05), DBP (r = 0.30; P < 0.05), Cre (r = 0.25; P < 0.05), eGFR (r = 0.35; P < 0.05), or TG (r = 0.29; P < 0.05). No correlation was observed between MG and the other parameters at baseline. Specifically, MG showed no significant correlation to markers for oxidative stress (8-OhdG, 8-epi-prostaglandin F2α, and pentosidine), inflammation (MCP-1, IL-6, IL-18, high-sensitive C-reactive protein, and TNFα), or macroangiopathy (IMT, PWV, and ABI). Unexpectedly, MG was not significantly correlated with either clinical parameters linked to diabetic macroangiopathy or clinical parameters linked to microangiopathy (ACR) or to biomarkers of oxidative stress or inflammation. This shows that MG was not directly involved in oxidative stress, inflammation, or arteriosclerosis of these patients at the time of the baseline examination. In addition, this may, at least in part, reflect the fact that the patients in our study had already taken a variety of drugs at the initiation of this study that might have influenced the progression of angiopathy, oxidative stress, and inflammation, such as renin-angiotensin system inhibitors (RASIs; n = 34), biguanide (n = 28), and statins (n = 14). On the other hand, at the baseline, 16 subjects were found to be RASI (−), 22 to be biguanide (−), and 5 to be both RASI (−) and biguanide (−). At the follow-up 5 years later, 7 subjects were RASI (−), 14 were biguanide (−), and none were both RASI (−) and biguanide (−). All of the subjects had been following a modified diet and an exercise regimen before taking part in this study, and there were no changes in these efforts during the test period. Despite the variety of treatments, the patients progressively developed hypertension and renal cardiovascular complications over the next 5 years: SBP increased from 138.7 ± 2.7 to 141.8 ± 2.7 mm Hg, IMT from 1.51 ± 0.12 to 1.60 ± 0.12 mm, PWV from 1725.4 ± 52.2 to 1792.7 ± 53.1 cm/s, and ACR from 126.4 (range: 2.7 to 3060.6) to 468.1 (4.7 to 4696.4) mg/g of Cre, and eGFR was decreased from 68.12 ± 4.37 to 49.04 ± 3.43 mL/min. We assessed the correlations between the baseline levels of MG or DG and the percentage changes after 5 years in IMT, PWV, SBP, and ACR or eGFR. The values of IMT, PWV, SBP, and eGFR all increased during the 5-year period. MG correlated significantly with the percentage of changes of IMT (Figure S4), SBP (Figure S5), eGFR (Figure S6), and PWV (Figure S7). The percentage of increase of SBP correlated significantly
with the percentage of increase of PWV (Figure S8). Please see the online Data Supplement.

As summarized in Table 2, MG showed a statistically significant correlation with all of these parameters. By contrast, DG was significantly correlated only with the percentage of change in ACR. However, the multiple regression analysis showed that MG was an independent risk factor for the percentage change of IMT, PWV, and SBP but not for that of ACR and eGFR (Table 3). DG was an independent risk factor for the percentage change of ACR (Table 3).

### Table 2. The Correlation Between the Percentage Changes During the 5-Year Period of IMT, PWV, SBP, ACR, and eGFR and the MG and DG (Using Simple Linear Regression Analysis)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Dependent</th>
<th>Independent</th>
<th>$R^2$</th>
<th>$r$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>% change of IMT</td>
<td>MG</td>
<td>0.3932</td>
<td>0.6271</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td></td>
<td>DG</td>
<td>0.0803</td>
<td>0.2835</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>% change of PWV</td>
<td>MG</td>
<td>0.3246</td>
<td>0.5697</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td></td>
<td>DG</td>
<td>0.001</td>
<td>0.0316</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>% change of ACR</td>
<td>MG</td>
<td>0.0979</td>
<td>0.3129</td>
<td>&lt;0.05</td>
<td></td>
</tr>
<tr>
<td></td>
<td>DG</td>
<td>0.1787</td>
<td>0.4227</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>% change of SBP</td>
<td>MG</td>
<td>0.257</td>
<td>0.5069</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td></td>
<td>DG</td>
<td>0.0013</td>
<td>−0.0363</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>% change of eGFR</td>
<td>MG</td>
<td>0.1294</td>
<td>0.3597</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td></td>
<td>DG</td>
<td>0.0061</td>
<td>0.0781</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

NS indicates not significant.

### Table 3. A Multiple Regression Analysis, Using the Percentage Changes in IMT, PWV, ACR, SBP, and eGFR Over the 5-Year Period as the Dependent or Objective Variables and the Values of MG, DG, HbA1c, TG, BMI, DD, and SBP at the Baseline as the Independent Variables

<table>
<thead>
<tr>
<th>Dependent Variable</th>
<th>Independent Variable</th>
<th>MG</th>
<th>DG</th>
<th>HbA1c</th>
<th>TG</th>
<th>BMI</th>
<th>DD</th>
<th>SBP</th>
</tr>
</thead>
<tbody>
<tr>
<td>% change of IMT</td>
<td>$\beta$</td>
<td>0.21</td>
<td>0.02</td>
<td>1.16</td>
<td>0.04</td>
<td>−0.06</td>
<td>0.04</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>95% CI</td>
<td>0.11</td>
<td>−0.01</td>
<td>−2.57</td>
<td>−0.02</td>
<td>−1.19</td>
<td>−0.48</td>
<td>−0.23</td>
</tr>
<tr>
<td></td>
<td>$P$</td>
<td>&lt;0.01</td>
<td>0.12</td>
<td>0.53</td>
<td>0.17</td>
<td>0.92</td>
<td>0.89</td>
<td>0.91</td>
</tr>
<tr>
<td>% change of PWV</td>
<td>$\beta$</td>
<td>0.08</td>
<td>−0.00</td>
<td>−0.26</td>
<td>0.00</td>
<td>−0.11</td>
<td>−0.23</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>95% CI</td>
<td>0.03</td>
<td>−0.01</td>
<td>−1.84</td>
<td>−0.02</td>
<td>−0.59</td>
<td>−0.45</td>
<td>−0.22</td>
</tr>
<tr>
<td></td>
<td>$P$</td>
<td>&lt;0.01</td>
<td>0.14</td>
<td>1.31</td>
<td>0.03</td>
<td>0.36</td>
<td>0.01</td>
<td>0.31</td>
</tr>
<tr>
<td>% change of SBP</td>
<td>$\beta$</td>
<td>0.13</td>
<td>−0.01</td>
<td>−0.64</td>
<td>−0.02</td>
<td>−0.05</td>
<td>−0.02</td>
<td>−0.20</td>
</tr>
<tr>
<td></td>
<td>95% CI</td>
<td>0.09</td>
<td>−0.02</td>
<td>−2.21</td>
<td>−0.04</td>
<td>−0.53</td>
<td>−0.24</td>
<td>−0.30</td>
</tr>
<tr>
<td></td>
<td>$P$</td>
<td>&lt;0.01</td>
<td>0.17</td>
<td>0.94</td>
<td>0.01</td>
<td>0.43</td>
<td>0.20</td>
<td>0.10</td>
</tr>
<tr>
<td>% change of ACR</td>
<td>$\beta$</td>
<td>4.03</td>
<td>1.37</td>
<td>−134.86</td>
<td>−1.09</td>
<td>3.67</td>
<td>−14.28</td>
<td>2.31</td>
</tr>
<tr>
<td></td>
<td>95% CI</td>
<td>−0.73</td>
<td>0.26</td>
<td>−315.99</td>
<td>−4.12</td>
<td>−51.34</td>
<td>−39.49</td>
<td>−9.37</td>
</tr>
<tr>
<td></td>
<td>$P$</td>
<td>8.79</td>
<td>2.48</td>
<td>46.27</td>
<td>1.95</td>
<td>58.68</td>
<td>10.94</td>
<td>13.99</td>
</tr>
<tr>
<td>% change of eGFR</td>
<td>$\beta$</td>
<td>0.10</td>
<td>0.02</td>
<td>0.14</td>
<td>0.48</td>
<td>0.89</td>
<td>0.26</td>
<td>0.69</td>
</tr>
<tr>
<td></td>
<td>95% CI</td>
<td>−0.10</td>
<td>−0.01</td>
<td>0.03</td>
<td>−0.06</td>
<td>−0.36</td>
<td>−0.29</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>$P$</td>
<td>0.07</td>
<td>0.58</td>
<td>0.99</td>
<td>0.12</td>
<td>0.57</td>
<td>0.32</td>
<td>0.82</td>
</tr>
</tbody>
</table>

DD indicates diabetic duration.

### Discussion

This study provides for the first time evidence that MG is a predictor in type 2 diabetes mellitus of intima-media thickening, of vascular stiffening, and of elevation of SBP, suggesting its clinical usefulness as a biomarker for diabetic macroangiopathy. Postprandial hyperglycemia increases MG and advances macroangiopathy. Therefore, MG is believed to increase the subsequent advancement of macroangiopathy in type 2 diabetes mellitus, especially in patients who have postprandial hyperglycemia,\textsuperscript{15,16} Postprandial hyperglycemia-induced epigenetic changes and increased inflammatory subunit expression are prevented by reducing mitochondrial superoxide production or superoxide-induced α-oxoaldehydes, such as MG,\textsuperscript{22} These results highlight the dramatic and long-lasting effects that short-term hyperglycemic spikes can have on vascular cells and suggest that transient spikes of hyperglycemia may be HbA1c-independent risk factors for diabetic complications. Postprandial hyperglycemia increases MG in the vascular cell by suppressing glyoxylase 1, which degrades MG activity. A rise in MG appears to be closely related to the decreased activity of glyoxylase 1 in the vascular endothelium, and this is related to metabolic memory. However, our study did not evaluate this glyoxylase 1. In the future, evaluation of glyoxylase-1 activity will be necessary.

Moreover, by suppressing the increase of MG, biguanide suppresses the advancement of macroangiopathy. (Biguanide is believed to suppress macroangiopathy not by improving postprandial hyperglycemia but by suppressing the increase of MG itself.) Our contention that MG predicts the develop-
The effects of RASIs by subgrouping the patients. Mori et al. demonstrated that administration of MG induces a rise in BP in experimental animals, which is significantly suppressed by administration of angiotensin receptor blockers or N-acetyl cysteine (an antioxidant agent). In blood vessels under diabetic conditions, MG primarily accumulates in endothelial cells. We reported recently that MG increases oxidative stress in vascular endothelial cells and induces vascular disorders. MG indeed triggers IMT hypertrophy and PWV stiffness (PWV) and the increase of the vascular thickness (IMT). Moreover, MG increases the salt sensitivity. These are better explanations as to why the levels of MG should be lower in patients with and those without biguanide treatment (P = 0.658). One plausible explanation for this finding would be that the dose of biguanides administered to patients in Japan is suboptimal, at only 750 mg/d for metformin and only 150 mg/d for buforin.

MG has been shown to be an independent variable affecting the percentage of change in SBP. MG has indeed been linked to the progression of hypertension in diabetic models through increases in vascular resistance, insulin resistance, and salt sensitivity and by the retention of body fluid volume. Previous studies by us and others have demonstrated that administration of MG induces a rise in BP in Dahl salt-sensitive rats with a normal diet and renorenal injury in Dahl salt-sensitive rats with a normal diet. We reported previously that MG increases oxidative stress in vascular endothelial cells and induces vascular disorders. MG indeed triggers IMT hypertrophy and PWV increase by an increase in local AGEs and oxidative stress. MG is an independent risk factor of the increase of vascular stiffness (PWV) and the increase of the vascular thickness (IMT). Moreover, MG increases the salt sensitivity. These are better explanations as to why the levels of MG should predict systolic and not diastolic pressure. We reported previously that administration of MG induces a rise in BP, increases ACR, and expands renal sclerotic lesions, all of which can be significantly suppressed by administration of angiotensin receptor blockers. Treatment with RASIs therefore appears to limit the organ damage caused by MG, and it has been indicated that angiotensin receptor blocker prevents MG-induced apoptosis by inhibiting caspase 3 activation, which might explain at least in part the beneficial effects of the angiotensin receptor blocker against diabetes-related cardiovascular diseases.

In our present study, the majority of patients (34 of 50 at the baseline and 43 of 50 after 5 years) had taken RASIs, making it difficult to analyze the effects of RASIs by subgrouping the patients. Mori et al. observed previously that MG induces hypertension and cardiorenal injury in Dahl salt-sensitive rats with a normal diet through the angiotensin II–mediated oxidative stress pathway. They found that, in the MG-treated rats, enhanced renal expressions of Ne-carboxyethyl-lysine (an AGE), 8-OHdG (a marker of oxidative stress), ED-1–positive cells (a marker of inflammation), and NAD(P)H oxidase activity occurred in parallel with a rise in SBP. However, in our study, the level of AGEs (eg, Ne-carboxyethyl-lysine) was not measured, so it is unclear whether MG directly causes vascular injury or whether MG changes into AGEs and causes vascular injury. An analysis that measures both AGEs and MG will be needed.

In the present study, we did not investigate the association of plasma levels of DG with diabetic retinopathy or neuropathy. It would be of interest to examine in a future study whether DG could be a useful biomarker for diabetic microangiopathy. MG is produced not only by hyperglycemia but also by a variety of proteins and conditions, such as TG. It accumulates inside the vascular endothelial cells, where it induces primarily macroangiopathy. On the other hand, DG is produced primarily by hyperglycemia and induces microangiopathy. We believe that this fact may explain why blood glucose improvement suppresses microangiopathy but not macroangiopathy. Despite an early loss of glycemic differences, a continued reduction in microvascular risk and emergent risk reductions for myocardial infarction and death from any cause were observed during 10 years of posttrial follow-up in the United Kingdom Prospective Diabetes Study. Early and rigorous blood glucose control thus has either a metabolic memory effect or a legacy effect of suppressing the onset of vascular disorders for extended periods. The possible mechanism of such effects remains unclear, although Holman et al. suggested that increased formation of AGEs may play an underlying role. The increased levels of MG observed in individuals with diabetes mellitus are not merely the result of short-term changes in glucose or MG but may reflect long-term alterations to tissue proteins.

In this context, it is of interest that MG, a precursor for AGEs, at the baseline is an independent risk factor for the percentage changes after 5 years of IMT, PWV, and BP. MG could be a target for future study to elucidate the biochemical mechanisms of such a legacy effect.

Perspectives

This study provides evidence that MG is a predictor of intima-media thickening, vascular stiffening, and elevation of BP in type 2 diabetics, suggesting its clinical usefulness as a biomarker for diabetic macroangiopathy. Medical agents interfering with α-oxoaldehydes, such as MG, or decreasing their production may have potential therapeutic benefits for diabetic angiopathies.

Limitations

This is an observational study in which diverse interventions and treatments were carried out over a 5-year period and, thus, cannot directly verify the cause-effect relationship of MG in the development of diabetic angiopathy. Therefore, there is a possibility that numerous factors have been modified. Because this study has numerous factors (eg, oxidative stress and inflammations markers) that were not measured after that period, this analysis is insufficient.

It is possible that higher levels of MG simply reflect lesser renal excretion of this substance. In fact, the plasma
levels of creatinine were correlated with MG at the onset of this study. Because urinary MG levels were not measured in this study, the possibility of a rise in MG levels being caused by a decline in renal function cannot be ruled out.

Acknowledgments

This study was conducted based on the formal contract of a cooperative study between Tohoku University and Nihon Trim (Trim Medical Institute Co., Ltd). We thank Manami Shimizu and Mai Sasaki for expert assistance with management of blood and urine samples.

Sources of Funding

This work was supported by the 21st Century Center of Excellence Program Special Research Grant from the Ministry of Education, Sports, and Culture and a research grant for cardiovascular research (19C-021) from the Ministry of Health, Labor, and Welfare of Japan (22C-005). Longitudinal/Cross-Sectional Studies to Generate Evidence for Diagnosis/Management of Metabolic Syndrome to be Used for Health Guidance (H19-SeiShu-001), Longitudinal/Cross-Sectional Studies to Generate Evidence for Diagnosis/Management of Metabolic Syndrome in Governmental Health Checkup and Guidance System (H22-SeiShu-005).

Disclosures

None.

References

Methylglyoxal Is a Predictor in Type 2 Diabetic Patients of Intima-Media Thickening and Elevation of Blood Pressure

Susumu Ogawa, Keisuke Nakayama, Masaaki Nakayama, Takefumi Mori, Masato Matsushima, Masashi Okamura, Miho Senda, Kazuhiro Nako, Toshio Miyata and Sadayoshi Ito

Hypertension. 2010;56:471-476; originally published online July 19, 2010;
doi: 10.1161/HYPERTENSIONAHA.110.156786

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2010 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/56/3/471

Data Supplement (unedited) at:
http://hyper.ahajournals.org/content/suppl/2010/07/16/HYPERTENSIONAHA.110.156786.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/
Methylglyoxal is a predictor in type 2 diabetic patients of intima-media thickening and elevation of blood pressure

Supplementary Figure

S1: Simple calibration curves for methylglyoxal (MG) and 3-deoxyglucosone (DG) assay. Regression equations of the standard curves were $y=21.886x+0.1532$ ($r=0.999$) and $y=3.1635x-0.0196$ ($r=0.997$) for MG and DG, respectively. Each sample was evaluated three times.
S2: Correlation between peak area ratio and concentration of MG and DG added to three plasma samples obtained from three different healthy controls. Regression equations for MG obtained from subject 1, 2 and 3 were $y=9.6267x+1.0232 \ (r=0.999)$, $y=9.0468x+1.4169 (r=0.999)$ and $y=9.2006x+1.2523 \ (r=0.999)$, respectively. Those for 3DG were $y=2.5219x+1.0209 \ (r=0.999)$, $y=2.5714x+1.2922 \ (r=0.999)$ and $y=2.3623x+1.4629 \ (r=0.994)$, respectively.

![Graph showing correlation between peak area ratio and concentration of MG and DG](image-url)
S3: Correlation between values obtained with previous and modified methods for MG assay.

Twenty dialysis patients and ten healthy controls were evaluated. Regression equation was

\[ y = 0.8934x - 92.02 \]

and correlation efficient was 0.987.
Supplementary Figure

The values of intima-media thickness (IMT), systolic blood pressure (SBP) increased, and estimated glomerular filtration rate (eGFR), decreased during the 5-year period. Methylglyoxal (MG) correlated significantly to the % changes of IMT (S4): $y = 0.2449x - 48.684$, $r = 0.6271$, $p < 0.01$, SBP (S5): $y = 0.0929x - 19.081$, $r = 0.5069$, $p < 0.01$, and eGFR (S6): $y = -0.1302x + 1.9887$, $r=0.3597$, $p < 0.01$. 

![Plasma MG levels vs % changes of IMT during 5 years](image)
S5

% changes of SBP during 5 years (%)

Plasma MG levels at baseline (nM)
Plasma MG levels at baseline (nM)

% changes of eGFR during 5 years (%)

S6
Supplementary Figure

The values of pulse wave velocity (PWV), systolic blood pressure (SBP) increased during the 5-year period. Methylglyoxal (MG) correlated significantly to the percent changes of PWV (S7): $y = 0.0892x - 16.296, r = 0.5697, p < 0.01$, percent change of SBP correlated significantly to the percent changes of PWV (S8): $y = 0.3092x + 3.7201, r = 0.3617, p < 0.01$. 

![Graph showing the correlation between plasma methylglyoxal level (nM) and percent changes of pulse wave velocity (%). The equation $y = 0.0892x - 16.296, r = 0.5697, p < 0.01$ is used to express the relationship.]