Short Report

Elevated level of methylglyoxal during diabetic ketoacidosis and its recovery phase

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SUMMARY
Carbonyl stress is hypothesized to be an associated complication of diabetic ketoacidosis. The production of the glycolytic intermediate methylglyoxal (MG) was followed up in 7 diabetic patients treated for ketoacidosis during pretreatment and recovery phase. Blood samples for methylglyoxal analysis were collected upon patient arrival in emergency department (0 h), and during ketoacidosis treatment between 12-24 h and at 168 h. The study also included 10 normoglycemic healthy volunteers and 31 type 1 diabetic patients (control diabetes group). The methylglyoxal assay, based on methylglyoxal derivation with 1,2-diamino-4,5-dimethoxybenzene (DDB), was performed by HPLC, only assessing the level of free methylglyoxal. The baseline level of methylglyoxal recorded in normoglycemic healthy controls was 338 ± 62 nmol/l versus 374 ± 89 nmol/l in control diabetes group (P = 0.0407). A consistent feature of diabetic ketoacidosis before and during treatment was striking elevation of methylglyoxal as compared with control diabetes group (median test \( \chi^2 = 14.6, df = 3, P = 0.0021 \)). Friedman’s ANOVA indicated differences (P = 0.04) among the three sampling times with a peak value (601 ± 95 nmol/l) at 12-24 h following therapy initiation. However, fasting treatment values at 168 h were still significantly higher than the mean fasting methylglyoxal level in control diabetes group (P = 0.008). The study indicated that diabetic ketoacidosis results in an increase in methylglyoxal level. Excessive production of toxic intermediates such are \( \alpha \)-dicarbonyls may be a link connecting an acute metabolic event with accelerated tissue damage, a feature characteristic of long-term complications of diabetes.

Key-words: Diabetes · Ketoacidosis · Carbonyl stress · Methylglyoxal.

RÉSUMÉ
Élévation des concentrations plasmatiques en méthylglyoxal au cours de l’acidocétose diabétique
La production du méthylglyoxal (MG), composé \( \alpha \)-dicarbonylé impliqué dans la survenue des complications du diabète, a été suivie chez sept patients diabétiques traités pour acidocétose. Les échantillons de sang pour l’analyse du méthylglyoxal ont été recueillis à l’admission et pendant le traitement de l’acidocétose entre 12-24 h et à 168 h. Dix volontaires normoglycémiques et 31 diabétiques de type 1 (groupe témoin diabétique) ont été également prélevés à jeun. Le dosage du méthylglyoxal, fondé sur la dérivation du méthylglyoxal avec 1,2-diamino-4,5-diméthoxybenzène (DDB), a été réalisé par HPLC. En conditions basales, les concentrations plasmatiques du méthylglyoxal étaient 338 ± 62 nmol/l chez les volontaires normoglycémiques versus 374 ± 89 nmol/l dans le groupe témoin diabétique (P = 0.0407). Une augmentation importante du méthylglyoxal a été observée chez les patients en acidocétose à l’admission et pendant le traitement (comparaison avec le groupe témoin : test médian \( \chi^2 = 14.6, df = 3, P = 0.0021 \)). Le test ANOVA de Friedman a montré des différences (P = 0.04) entre les valeurs des trois temps d’échantillonnage avec une valeur maximale (601 ± 95 nmol/l) 12-24 h après le début du traitement. Les concentrations plasmatiques à jeun après 168 h de traitement étaient encore significativement plus élevées que celles du groupe témoin diabétique (P = 0.008). Cette étude montre que l’acidocétose diabétique s’accompagne d’une augmentation des concentrations plasmatiques en méthylglyoxal. L’accentuation de la production de dérivés carbonylés au cours des épisodes d’acidocétose pourrait être impliquée dans la pathogénie des complications des sujets diabétiques de type 1.

Mots-clés : Diabète · Acidocétose · Stress carbonylé · Méthylglyoxal.

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Recent research supports an important role of reactive \(\alpha\)-dicarbonyl compounds as intermediates in post-translational modification of proteins, and advanced glycation end-product (AGE) formation [1]. It has been shown that the formation of AGEs in vivo contributes to the pathophysiological changes associated with aging and long-term complications of diabetes [2]. Glycation of proteins is enhanced during hyperglycaemia, both because of increased glucose per se and alterations in cellular metabolism leading to elevated production of reactive \(\alpha\)-dicarbonyls. These compounds are implicated in the development of a number of pathologies via a condition known as “carbonyl stress”. Among them, methylglyoxal, glyoxal and 3-deoxyglucosone are of particular interest in diabetes [3,4].

Diabetic ketoacidosis is acute hyperglycaemic emergency, which can occur in both type 1 and type 2 diabetes. The basic underlying mechanisms are decreased effective action of insulin and elevation of counter-regulatory hormones. Both disorders lead to derangement of electrolyte and mineral metabolism with severe alteration of lipid, carbohydrate and protein metabolism. It has been hypothesized that metabolic crisis such as diabetic ketoacidosis may cause overproduction of reactive \(\alpha\)-dicarbonyl compounds [5] and concomitantly accelerate damaging of biological macromolecules. Considering the potential toxicity of \(\alpha\)-dicarbonyls, the production of methylglyoxal was followed up during pretreatment and recovery phases of diabetic ketoacidosis.

Patients and methods

Patients

Seven diabetic patients with ketoacidosis, ten normoglycaemic volunteers, and 31 type 1 diabetic patients (control diabetes group) with median HbA\(_1c\) at 10.4% (range 5.02-13.4%) were included in the study. Five patients with ketoacidosis had previously diagnosed diabetes and two patients were newly diagnosed diabetics. The previously diagnosed patients were on insulin regimen, median disease duration 4 (range 3-17) years, and had received education on diabetic diet. The ketoacidosis treatment included correction of dehydration, hyperglycaemia and electrolyte imbalance, followed by identification of comorbid precipitating conditions and careful monitoring of patient cardiac, renal, metabolic and mental status [6]. Fluid replacement therapy corrected the estimated deficits within the first 24 h and consisted of isotonic saline (0.9% sodium chloride) infused at approximately 1-1.5 L during the first hour, followed by 0.9% NaCl or 0.45% KCl, depending on serum sodium (normal or elevated), infused at 4 to 14 ml/kg/h. Hyperglycaemia was corrected by continuous infusion of regular insulin at an average dose of 6 U/h. Blood was sampled every 2-4 h for determination of plasma glucose, electrolytes, blood urea nitrogen, creatinine, osmolality, and venous pH. When plasma glucose reached 14 mmol/l, 5% glucose was added to intravenous fluid. Once ketoacidosis was resolved (plasma glucose < 11 mmol/l, serum bicarbonate ≥ 18 mEq/l, venous pH > 7.3) and the patient was able to eat, subcutaneous regular insulin was administered every 6 h to control plasma glucose.

Samples for methylglyoxal analysis were collected upon patient arrival in emergency department (0 hour, \(t_0\), and during treatment between 12-24 h \((t_1)\) and at 168 h \((t_2)\). Fresh blood samples were collected into sterile tubes containing EDTA, Na, and 1.0 ml of whole blood was immediately injected into a vial containing 100 \(\mu\)l of trifluoroacetic acid (TFA), vigorously vortexed, and stored at -20°C.

The reported investigation was designed and carried out in accordance with the principles of the Declaration of Helsinki as revised in 2000, and was approved by the Ethics Committee of the University Clinic for Diabetes, No. 04-2029.

Methylglyoxal assay

The reversed-phase high-performance liquid chromatography method (RP HPLC) with UV detection was used to detect unbound methylglyoxal (only free form) in blood samples. The processing of samples required protein precipitation with trifluoroacetic acid (TFA), incubation of the supernatant (2 h) with 1,2-diamino-4,5-dimethoxybenzene (DDB) to convert methylglyoxal to 6,7-dimethoxy-2-methylquinoxaline (DMQ), freeze-drying, and RP HPLC analysis using 6,7-dimethoxy-2,3-dimethylquinoxaline (DMDQ) as an internal standard. Simplified methods for the synthesis of methylglyoxal and DDB have been previously described [7]. Calibration curves were linear in the range of 200-1000 nmol/l. The limit of detection was 30.6 and 45.9 pmol, at 215 and 352 nm, respectively. The inra-day coefficients of variation were 6.9-12.6% for 215 nm and 3.5-12.6 for 352 nm. The inter-day coefficients of variation were 9.6-12.8 for 215 nm and 7.2-14.7% for 352 nm.

RP HPLC was performed on an Äkta Purifier HPLC system (Pharmacia Biotech, Uppsala, Sweden) with P-900 pumps, UV-900 spectrophotometer, flow cell with path length of 10 mm and A-900 autosampler) with an Eurosep 100 reversed-phase C-18 column (150 x 4.0 mm I.D., 5 \(\mu\)m) (Knauer, Bad Homburg, Germany).

Results

Table I shows pretreatment metabolic values in study subjects. Blood was drawn on admission for diagnosis and clinical management of diabetic ketoacidosis. On admission, patients with DKA had a mean plasma glucose of 25.9 ± 8.9 mmol/l, capillary blood pH < 7.34, bicarbonate < 13.5 mmol/l, osmolality > 280 mOsm/kg, and positive ketonuria. In addition, the biogenesis of methylglyoxal
(MG) was monitored and MG level was quantified by HPLC as a free MG compound (not adducts of MG). The levels of methylglyoxal during pretreatment and recovery from ketoacidosis are shown in figure 1. Methylglyoxal measured in blood samples of control diabetes group (374 ± 89 nmol/l, n = 31) showed a scatter of individual values and confidence interval ± 95% for the mean ranging from 335 to 411 nmol/l (figure 1). The baseline level of methylglyoxal recorded in normoglycaemic controls (n = 10) was 338 ± 62 nmol/l. A consistent feature of diabetic ketoacidosis before and during treatment was a striking elevation of methylglyoxal as compared with control diabetes group (median test \( \chi^2 = 14.6, df = 3, P = 0.0021 \)). Friedman’s ANOVA indicated differences (p = 0.04) among the three sampling times with a peak value (601 ± 95 nmol/l) at 12-24 h of therapy initiation. However, fasting treatment values at 168 h were still significantly higher than the mean fasting methylglyoxal level in the control diabetes group (P = 0.008). During pretreatment and recovery from ketoacidosis there was no correlation between methylglyoxal and blood glucose. Figure 2 shows the sustained methylglyoxal elevation in relation to plasma glucose at three sampling times. Contrary to this, in the control diabetes group blood glucose showed a significant but inverse correlation with the concentration of methylglyoxal (r = -0.46, \( P = 0.0073 \)). Regression analysis showed no significant impact of pH (capillary blood) (P = 0.5); HCO\(_3\) (P = 0.4); base excess (P = 0.35); pCO\(_2\) (P = 0.65); tCO\(_2\) (P = 0.39); pO\(_2\) (P = 0.7); O\(_2\) SAT (P = 0.8); Na\(^+\) (P = 0.06); K\(^+\) (P = 0.7); L-lactate (P = 0.76); osmolality (P = 0.83); and creatinine (P = 0.39) on the methylglyoxal level.

### Discussion

The present study revealed that diabetic ketoacidosis, an acquired metabolic disorder, could produce a considerable amount of methylglyoxal, which may be of pathologic significance in the development of diabetic complications. Methylglyoxal (measured as a free, unbound compound) was monitored and MG level was quantified by HPLC as a free MG compound (not adducts of MG). The levels of methylglyoxal during pretreatment and recovery from ketoacidosis are shown in figure 1. Methylglyoxal measured in blood samples of control diabetes group (374 ± 89 nmol/l, n = 31) showed a scatter of individual values and confidence interval ± 95% for the mean ranging from 335 to 411 nmol/l (figure 1). The baseline level of methylglyoxal recorded in normoglycaemic controls (n = 10) was 338 ± 62 nmol/l. A consistent feature of diabetic ketoacidosis before and during treatment was a striking elevation of methylglyoxal as compared with control diabetes group (median test \( \chi^2 = 14.6, df = 3, P = 0.0021 \)). Friedman’s ANOVA indicated differences (p = 0.04) among the three sampling times with a peak value (601 ± 95 nmol/l) at 12-24 h of therapy initiation. However, fasting treatment values at 168 h were still significantly higher than the mean fasting methylglyoxal level in the control diabetes group (P = 0.008). During pretreatment and recovery from ketoacidosis there was no correlation between methylglyoxal and blood glucose. Figure 2 shows the sustained methylglyoxal elevation in relation to plasma glucose at three sampling times. Contrary to this, in the control diabetes group blood glucose showed a significant but inverse correlation with the concentration of methylglyoxal (r = -0.46, \( P = 0.0073 \)). Regression analysis showed no significant impact of pH (capillary blood) (P = 0.5); HCO\(_3\) (P = 0.4); base excess (P = 0.35); pCO\(_2\) (P = 0.65); tCO\(_2\) (P = 0.39); pO\(_2\) (P = 0.7); O\(_2\) SAT (P = 0.8); Na\(^+\) (P = 0.06); K\(^+\) (P = 0.7); L-lactate (P = 0.76); osmolality (P = 0.83); and creatinine (P = 0.39) on the methylglyoxal level.

### Table I

Pretreatment metabolic parameters.

<table>
<thead>
<tr>
<th></th>
<th>Case 1</th>
<th>Case 2</th>
<th>Case 3</th>
<th>Case 4</th>
<th>Case 5</th>
<th>Case 6</th>
<th>Case 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mmol/l)</td>
<td>12.8</td>
<td>28.8</td>
<td>32.9</td>
<td>22.4</td>
<td>16.4</td>
<td>31.8</td>
<td>36.4</td>
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<tr>
<td>HCO(_3) (mmol/l)</td>
<td>8.5</td>
<td>2.9</td>
<td>9.0</td>
<td>13.4</td>
<td>7.3</td>
<td>4.7</td>
<td>12.6</td>
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<tr>
<td>Base excess (mmol/l)</td>
<td>-17.3</td>
<td>-29.9</td>
<td>-17.2</td>
<td>-8.4</td>
<td>-20.0</td>
<td>-26.6</td>
<td>-13.8</td>
</tr>
<tr>
<td>pCO(_2) (KPa)</td>
<td>2.97</td>
<td>1.81</td>
<td>3.03</td>
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<td>2.72</td>
<td>2.54</td>
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<td>TCO(_2) (mmol/l)</td>
<td>9.2</td>
<td>3.3</td>
<td>9.7</td>
<td>15.2</td>
<td>7.9</td>
<td>5.3</td>
<td>13.4</td>
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<tr>
<td>O(_2) SAT (%)</td>
<td>6.8</td>
<td>7.94</td>
<td>8.23</td>
<td>9.71</td>
<td>7.46</td>
<td>7.28</td>
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<td>O(_2) SAT (%)</td>
<td>77</td>
<td>77.9</td>
<td>88</td>
<td>94.5</td>
<td>83.1</td>
<td>74.9</td>
<td>94.5</td>
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<td>Na(^+) (mmol/l)</td>
<td>132</td>
<td>130</td>
<td>146</td>
<td>136</td>
<td>142</td>
<td>140</td>
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<tr>
<td>K(^+) (mmol/l)</td>
<td>3.2</td>
<td>5.7</td>
<td>4.4</td>
<td>3.9</td>
<td>5.4</td>
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<tr>
<td>L-lactate (mmol/l)</td>
<td>1.47</td>
<td>3.7</td>
<td>0.96</td>
<td>1.48</td>
<td>2.37</td>
<td>2.86</td>
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<td>Osmolality (mOsm/kg)</td>
<td>285</td>
<td>340</td>
<td>360</td>
<td>320</td>
<td>340</td>
<td>335</td>
<td>315</td>
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<tr>
<td>Creatinine (µmol/l)</td>
<td>91</td>
<td>166</td>
<td>180</td>
<td>233</td>
<td>128</td>
<td>119</td>
<td>165</td>
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<tr>
<td>Urine ketones (yes/no)</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
</tr>
</tbody>
</table>

Figure 1

Methylglyoxal level measured in whole blood samples before (t\(_0\) = 0 h) and during (t\(_1\) = 12-24 h, t\(_2\) = 168 h) treatment in patients with diabetic ketoacidosis (n = 7).
Methylglyoxal is an intermediate in the glycolytic pathway, and consequently rises from an increased flux during hyperglycaemia. In our study, the treatment for ketoacidosis and glycaemia control failed to produce a significant reduction of methylglyoxal toward control values of diabetic population in general. This observation suggests the necessity of analyzing other possible sources of methylglyoxal. Although the biogenesis of methylglyoxal occurs primarily from the triose phosphate intermediates, other sources of its formation include ketone body metabolism from acetone. In acute diabetic ketoacidosis, the ketone body ratio of β-hydroxybutyrate to acetoacetate rises from normal (1:1) to as high as 10:1. With therapy, β-hydroxybutyrate is converted to acetoacetate and ketosis appears to worsen. Because it is a β-keto acid, acetoacetate also undergoes a slow spontaneous decarboxylation to acetone. Methylglyoxal can be derived from both acetoacetate and acetone. Its production from acetate is mediated by myeloperoxidase, whereas P450 2E1 cytochrome catalyzes methylglyoxal generation from acetone. Unfortunately, we were not able to perform quantitative measurements of ketone bodies. However, the fact that no correlation was found between methylglyoxal and blood glucose during pretreatment and recovery of patients with ketoacidosis is an argument for involvement of ketone bodies in the overproduction of methylglyoxal. Although further studies would be necessary to assess the contribution of the acetone-glucose pathway to the increased methylglyoxal production, our study is supported by a recent report by Beisswenger et al. [13].

Apart from the ketone body pathway, the sustained increase in the level of methylglyoxal may be the consequence of decreased clearance of this compound by detoxification pathways. Methylglyoxal detoxification is mostly catalyzed by the glyoxalase system, leading to the production of the inert end product D-lactate. Optimal activity of the glyoxalase system is dependent on adequate levels of reduced glutathione (GSH). Hyperglycaemia and oxidative stress, both of which are increased in ketoacidosis, are associated with GSH depletion. Thus, impaired detoxification in relation with depletion in reduced glutathione may be hypothesized to account for the observed evolution of methylglyoxal. Determination of D-lactate in future studies could strengthen this hypothesis.

In conclusion, excessive production of dicarbonyls during ketoacidosis can enhance post-translational protein modification, suggesting that ketotic incidents might accelerate the pathophysiological process leading to tissue damage, a pattern characteristic of chronic diabetes complications. Therefore, the present study suggests that metabolic crisis of ketoacidosis has relevance with regard to the pathogenesis of both acute and long-term complications of diabetes.

**References**


**Figure 2**

Comparison of methylglyoxal level and blood glucose measured before and during the treatment of ketoacidosis.


