SERUM LEVELS OF METHYLGLYOXAL AND CARBOXYMETHYL LYSINE ARE INCREASED IN PATIENTS WITH TYPE 1 DIABETES AND REDUCED KIDNEY FUNCTION

The FinnDiane Study

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OBJECTIVES

- Advanced glycation end-products (AGEs) are modified proteins formed through enzymatic and non-enzymatic degradation of glucose during hyperglycemic conditions (1,2)
- The reactive dicarbonyls methylglyoxal (MG), glyoxal and diacylglycerol (DG) are considered the most reactive AGE precursors.
- N_e-carboxymethyl lysine (CML) is a product of oxidative modification of glycated proteins and also a potent AGE
- Inhibition of AGE accumulation reduces the progression of atherogenesis and diabetic nephropathy in experimental models, while their role in clinical diabetes has not been established
- **The aim of this study was to determine the circulating levels of MG and CML-modified proteins in patients with type 1 diabetes (T1D) and their association with the severity of diabetic nephropathy**

METHODS

Serum MG and CML were measured in 350 adults with T1D and in 114 healthy control subjects as part of the Finnish Diabetic Nephropathy (FinnDiane) study (3). Diagnostic criteria for T1D include age of onset below 35, the transition to permanent insulin treatment within a year from onset and C-peptide negativity. Patients with end-stage renal disease (ESRD) were not included in this analysis. MG was determined by HPLC after derivatization with 1,2-diamino-4,5-dimethoxybenezene (4). CML was determined by an ELISA-based method. Glomerular filtration rate (GFR) was estimated using the CKD-EPI formula (5). AER was assessed from a 24-h urine collection by immunoturbidimetry. Differences between groups were analyzed with ANOVA. For between-group comparisons among the various groups, post-hoc tests were performed using Fisher's least significant difference (LSD) method. The age- and gender adjustment were performed by using age as a covariate in the univariate analysis. Categorical variables were analyzed using Pearson's χ^2 test. $P<0.05$ was considered statistically significant.

RESULTS

- Serum MG was higher in patients with T1D as compared to control subjects (411±8 vs 340±12 nM, $P<0.05$).
- Patients with reduced kidney function (GFR <30 ml/min per 1.73m²) had significantly higher MG levels than those with GFR >90 (476±32 vs 395±11 nM, $P<0.05$) (Figure 1).
- CML levels were higher in the T1D patients with GFR <30 than in those with GFR >90 (510±26 vs 459±7 nM, $P<0.05$). However, healthy control subjects had higher CML levels (482±7) than T1D patients with GFR >90. (Figure 2).
- GFR was negatively correlated with MG (r=-0.113, P=0.035) and CML (r=-0.121, $P<0.05$). These correlations remained statistically significant also after adjustment for age and gender.
- Urinary albumin excretion rate (AER), age, gender, or HbA_{1C} were not associated with serum MG or CML levels in T1D patients or in controls.

Table 1. Demographic and biochemical characteristics of the study subjects

	Type 1 diabetes	Healthy controls	*P*-value
n	350	114	
Male n (%)	176 (50)	79 (69)	<0.001
Age (years)	44.4±0.6	30.7±0.7	<0.001
Diabetes duration (years)	31.1±0.6	-	-
HbA_{1c} (%)	8.2±0.1	5.3±0.01	<0.001*
BMI (kg/m²)	26.5±0.2	24.2±0.3	<0.001*
Waist/Hip ratio	0.89±0.01	0.88±0.01	n.s.
SBP (mmHg)	141±1.0	129±1	<0.001*
DBP (mmHg)	78±0.5	77±1	n.s.
eGFR (ml/min/1,73m²)	86±2	104±2	<0.001*
AER (mg/24h)	450±75	19±2	0.001*
Total cholesterol (mmol/l)	4.8±0.1	4.5±0.1	<0.001
TG (mmol/l)	1.3±0.04	1.00±0.10	<0.001
HDL cholesterol (mmol/l)	1.5±0.02	1.7±0.05	<0.001
MG (nM)	411±8	340±12	<0.001*
CML (IU)	467±5	482±7	n.s.

*$P<0.05$ also with adjustment for age and gender. HbA_{1c}; hemoglobin A_{1c}, BMI; body mass index, SBP; systolic blood pressure, DBP; diastolic blood pressure, eGFR; estimated glomerular filtration rate, AER; albumin excretion rate, TG; triglycerides, MG; methylglyoxal, CML; carboxymethyl lysine. Data are presented as mean±SEM. n.s.; non significant

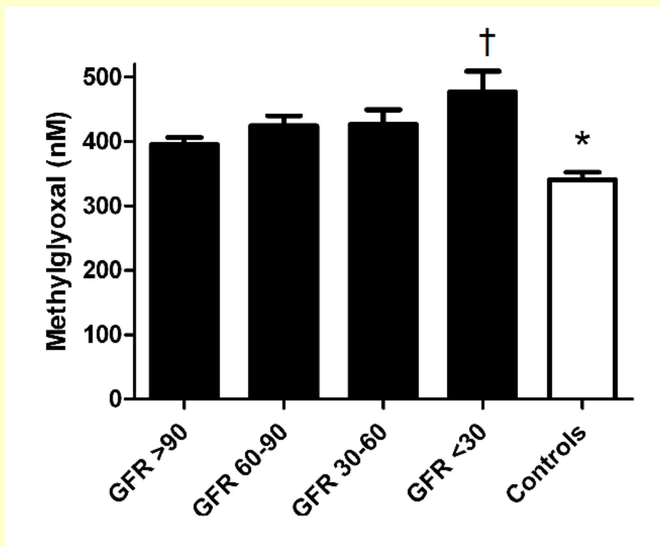

Figure 1. *$P<0.05$ between control group and subgroups of T1D patients , †$P<0.05$ between T1D patients with GFR >90 and GFR <30 ml/min per 1.73m² . Data presented as mean±SEM.

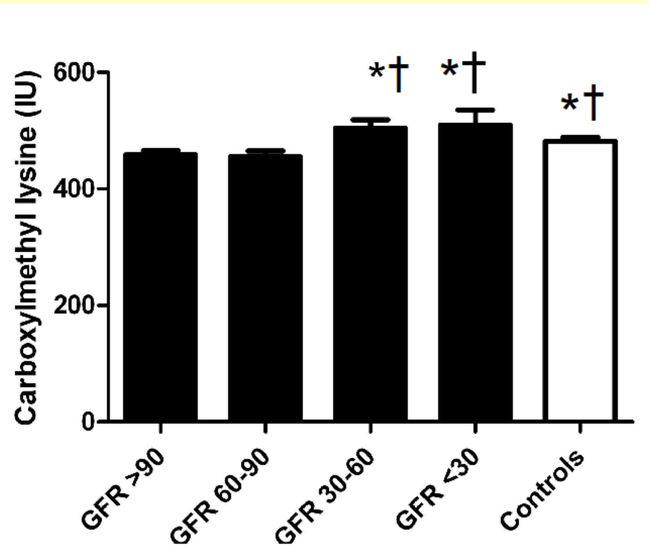

Figure 2. *$P<0.05$ when compared to T1D patients with GFR >90, †$P<0.05$ when compared to T1D patients with GFR 60-90, and <30 ml/min per 1.73m² . Data presented as mean±SEM.

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CONCLUSIONS

The pharmacologic approaches currently in clinical use have remained unsatisfactory in preventing the diabetic complications. Therefore, more innovative tactics are needed for the prevention and reversal of the vascular damage in diabetes. The deterioration of the kidney function was strongly associated with increased levels of MG and CML in patients with T1D. These results support the role of circulating AGEs in the development of vascular complications in diabetes.

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I) Diabetes. I2) Clinical studies.
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DOI: 10.3252/pso.eu.51era.2014
Poster presented at: 51 ERA-EDTA CONGRESS AMSTERDAM 2014