# Monitoring of early and advanced glycation in relation to the occurrence of microvascular complications in children and adolescents with type 1 diabetes mellitus.

Jana KOSTOLANSKÁ[1], Vladimír JAKUŠ[2], Ľubomír BARÁK[1]

[1]*Children Diabetological Center of the Slovak Republic, Department of Pediatrics, University Hospital, Faculty of Medicine, Comenius University, Bratislava*

[2]*Institute of Medical Chemistry, Biochemistry and Clinical Biochemistry, Faculty of Medicine, Comenius University, Bratislava*

Correspondence to: Dr. Vladimír Jakuš, PhD., Institute of Medical Chemistry, Biochemistry and Clinical Biochemistry, Faculty of Medicine, Comenius University, Sasinkova 2, 811 08 Bratislava

E-mail: vladimir.jakus@fmed.uniba.sk

The short tittle: Monitoring of serum AGEs

**Summary**

The authors aimed to evaluate if the monitoring of serum advanced glycation end products (s-AGEs) could help predict a development of diabetic complications. Clinical and biochemical parameters including fructosamine (FAM), glycated hemoglobin (HbA1c) and s-AGEs were investigated in children and adolescents with 1 type diabetes with (+DC) and without (–DC) complications. The FAM levels (in mmol/l) were significantly elevated in +DC diabetic group compared with –DC one (3.043±0.459 vs. 2.614±0.430; $p<<0.001$) and with controls (3.043±0.459 vs. 1.620±0.340; $p<<0.001$) and in –DC vs. controls too (2.614±0.430 vs. 1.620±0.340; $p<<0.001$). HbA1c (in %) were significantly elevated in +DC diabetic group compared with –DC one (10.48±1.83 vs. 8.41±1.19; $p<<0.001$) and with controls (10.48±1.83 vs. 5.0±0.38, $p<<0.001$) and also in –DC vs. controls (8.41±1.19 vs. 5.0±0.38; $p<<0.001$). The s-AGEs levels (in A. U.) were significantly higher in +DC group than in –DC (73.0±14.09 vs. 65.8±9.05; $p<0.05$) and in group +DC than in controls (73.0±14.09 vs. 60.17±13.78; $p<0.05$), whereas not in –DC vs. controls. FAM was correlated with HbA1c in both diabetic groups (in +DC: $r=0.374$; $p<0.05$; in –DC: $r=0.719$; $p<<0.001$), but not in controls. S-AGEs were correlated with HbA1c ($r=0.478$; $p=0.003$) in +DC, but not in –DC or controls. Enhanced s-AGEs levels show that they could be not only microangiopathies attendant phenomenon of, but also a predictor of their development.

Key words: fructosamine, HbA1c, s-AGEs, glycation gap, diabetic complications

**Introduction**

Hyperglycemia and poor glycemic control are considered to be the key basal factors in the development of diabetic complications. Acute and chronic hyperglycemia is known to enhance the forming of early (fructosamine (FAM), glycated hemoglobin (HbA1c)),

intermediate and advanced glycation products and is a primary factor that initiates and promotes diabetic complications (Little and Goldstein 1995, Hanssen 1997). Glycation has both physiological and pathophysiological significance (Rácz and Šipulová 1998, Jakuš and Rietbrock 2004).

FAM fraction reacts much more quickly than the HbA1c to a change in glucose situation and reflects a quality of diabetes control over the previous 2 – 3 weeks. FAM preceded an increase in albumin excretion rate and is associated with nephropathy and exerted a pathophysiological role in microvascular diabetic complications. The degree of glycation of hemoglobin provides information about the glucose level (quality of diabetes control) over the previous 6 – 8 weeks (Michalková et al. 1981, Rácz et al. 1989, Gugliucci 2000, Cohen et al. 2002).

Hyperglycemia accelerates also the formation of advanced glycation end products (AGEs). The AGEs are a heterogeneous group of fluorescent and non-fluorescent compounds including the following main subgroups: bis(lysyl)imidazolium cross-links, hydroimidazolones, 3-deoxyglucosone derivatives, and monolysyl adducts (Wautier and Schmidt 2004), the fluorescent compounds with an excitation maximum at 370 nm and emission at ~445 nm (Galler et al. 2003, Kumar et al. 2004). They can form in short- and long-lived proteins such as albumin, immunoglobulins, skin collagen, ocular, renal and vascular tissues (Thorpe and Baynes 2003, Januszewski et al. 2008). Many AGEs have not been well characterized, but a few AGE-structures have been identified. According to Januszewski et al. (2008) AGE levels may have a role in the identification of Type 1 diabetic patients at high risk for complications and also provide a tool for monitoring therapeutic interventions. Measurement of s-AGEs is hence of great importance for clinicians and researchers concerned with the management and prevention of diabetic vascular diseases (Sampathkumar et al. 2005). Cohen et al. (2003, 2008) have dealt with glycation gap (GG) of

HbA1c calculated from FAM and have found a relation of gap to nephropathy and retinopathy. We have dealt with not only glycation gap of HbA1c, but also with GG of s-AGEs from HbA1c.

The aim of the present study was to evaluate if monitoring of circulating FAM, HbA1c and s-AGEs in patients with type 1 diabetes could help to predict the diabetic microvascular complications development. We aimed also to find a relationship of GG of HbA1c and GG of s-AGEs to DC.

## Materials and methods

*Patients and the sample collection*

Blood, serum and urine were obtained from 76 diabetic patients regularly attending the 1st Department of Pediatrics, Children Diabetological Center of the Slovak Republic, University Hospital, Faculty of Medicine, Comenius University, Bratislava. Patients were from 7 to 18 years old except 2 subjects 19 years of age and have Type 1 diabetes with duration at least 5 years. There were observed no microvascular complications (nephropathy, retinopathy, neuropathy) in 35 subjects including both 19 year old patients (–DC), whereas 41 subjects were with complications (+DC). Urinary albumin excretion rate (UAER) in the microalbuminuric range and its tracking (i.e. annual increase) are still considered reliable markers for prediction of later overt diabetic kidney disease. Irrespective of the procedure used, at least two samples over a 3-6-month period should test positive before microalbuminuria is confirmed and 'persistent microalbuminuria' defined (Chiarelli et al. 1997). The urine samples were collected 3 times overnight, microalbuminuria was considered present when the UAER was between 20 and 200 μg/min (Mogensen 1995, Mojto and Tisoň 2004) in 2 samples (according to Galler et al. 2003). No changes (fundus diabetic retinopathy) were found by the ophtalmologist examining the eyes in subject without retinopathy. Diabetic

neuropathy was confirmed by EMG exploration using the conductivity assessment of sensor and motor fibres of peripheral nerves. 59 patients were of long time poor glycemic control (mean of HbA1c during last 2 years >8.5 %) and 52 of poor short time glycemic control (actual data of HbA1c>8.5 %). 30 children without diabetes or other metabolic disease from 0 to 17 years old were used as controls.

*Methods*

The values of FAM were determined by spectrophotometric method at wavelength 530 nm (LS 500, SRN). We used 1-deoxy-1-morpholino-fructose (Sigma Aldrich, USA) as the standard.

HbA1c was measured by LPLC (DiaSTAT, USA). The HbA1c values were obtained by the National Glycohemoglobin Standardization Program (NGSP) calibrated method (John et al. 2007). Values of total cholesterol (TC), high density lipoproteins (HDL) and triacylglyceroles (TAG) were evaluated enzymatically, low density lipoproteins (LDL) was obtained using Friedewald formula. UAER was determined turbidimetrically (Integra 400 Plus, Roche, Switzerland).

S-AGEs were determined as AGE-linked specific fluorescence, serum was diluted 20-fold with deionized water, the fluorescence intensity was measured after excitation at 346 nm, at emission 418 nm using a spectrophotometer Perkin Elmer LS-3, USA. Chinine sulphate (1μg/ml) (Merck, Germany) was used to calibrate the instrument. Fluorescence was expressed as the relative fluorescence intensity in arbitrary units (A.U.).

*Glycation gap*

According to Cohen et al. (2003, 2008) there was determined GG there. GG was defined as the difference between the measured HbA1c and HbA1c predicted from the FAM based on the HbA1c-FAM regression equation:

$$GG = (\text{HbA1c measured} - \text{HbA1c predicted})$$

There were used the FAM values of all diabetic subjects for calculating of predicted HbA1c values using the regression line equation. By the definition, GG is negative if measured HbA1c is less than HbA1c predicted from FAM and positive if measured HbA1c is greater than predicted. For example, HbA1c at the lower limit of normal and FAM above the upper limit of normal would result in a negative GG.

In this study the GG of HbA1c expressed in % was calculated using the formula:

$$GG = 100 \times (\text{HbA1c measured} - \text{HbA1c predicted}) / \text{HbA1c measured}$$

GG of s-AGEs was established. The expected values of s-AGEs were predicted from the actual HbA1c values and were based on the s-AGEs-HbA1c regression.

The GG was expressed in % using the formula:

$$GG = 100 \times (\text{s-AGEs measured} - \text{s-AGEs predicted}) / \text{s-AGEs measured}$$

*Statistics*

Unpaired Student´s t-test (by Excel 2000) and Pearson linear correlation (by Origin 3.83) were used for statistical calculation. $\chi^2$-tests were performed using Excel 2000. For a p value less than 0.05 the statistical significance was defined as significant.

**Results**

*Comparison of clinical and biochemical parameters*

The FAM levels are significantly higher in both diabetic groups in comparison with controls (+DC vs. controls: 3.043±0.459 mmol/l vs. 1.620±0.340 mmol/l, –DC vs. controls: 2.614±0.430 mmol/l vs. 1.620±0.340 mmol/l, in both $p<<0.001$) and in group +DC vs. -DC (FAM 3.043±0.459 mmol/l vs. 2.614±0.430 mmol/l; $p<<0.001$) (Table 1).

We observed significantly higher HbA1c levels in both diabetic groups in comparison with controls (+DC vs. controls: 10.48±1.83 % vs. 5.0±0.38 %, –DC vs. controls: 8.41±1.19 % vs.

5.0±0.38 %, in both p<<0.001) (Table 1) and in group +DC vs. –DC (10.48±1.83 % vs. 8.41±1.19 %, p<<0.001) (Table 1).

The s-AGEs levels were significantly higher in subjects +DC than in controls (73.0±14.09 A.U. vs. 60.17±13.78 A.U., p<0.001) (Table 1, Fig. 1), but the difference between –DC patients and controls was not significant. The levels of s-AGEs were also significantly higher in group +DC vs. –DC (73.0±14.09 A. U. vs. 65.8±9.05 A. U., p=0.02) (Fig. 1).

The HDL levels were significantly higher in all diabetic groups in comparison with controls (+DC vs. controls: p<<0.001, –DC vs. controls: p=0.02). Lower HDL levels in controls compared with diabetic patients could be explained by significantly lower age of controls.

The levels of TAG are lower in diabetic group -DC than in controls (p=0.03) and in -DC vs. +DC (p=0.003). The levels of creatinine were higher in all diabetic groups than in controls and the differences were extremely significant (+DC vs. controls: 59.1±11.33 µmol/l vs. 42.43±12.25 µmol/l, –DC vs. controls: 57.09±11.04 µmol/l vs. 42.43±12.25 µmol/l, p<<0.001).

*Correlations*

The significant correlations between creatinine and age were observed in diabetic subjects –DC (r=0.684, p<<0.001) and +DC too (r=0.542, p<<0.001) (Table 2). Creatinine was correlated with diabetes duration in both diabetic groups (–DC: r=0.407, p=0.02, +DC: r=0.452, p=0.003). TAG were closed correlated with age in subjects –DC (r=0.352, p=0.04) (Table 2), and with HbA1c in group +DC (r=0.433, p=0.005), HDL was correlated inversely with age in –DC group (r=-0.386, p=0.02). The s-AGEs were correlated with HbA1c (mean levels in previous 2 years) in subjects +DC (r=0.478, p=0.004) (Fig. 2), but not in –DC group and with FAM in group +DC (r=0.396, p=0.02). S-AGEs were significantly correlated also with actual HbA1c levels in +DC (r=0.366, p=0.03), but not as significantly as with mean levels, and not in –DC and controls. There were observed significant correlation between

HbA1c and creatinine (r=0.494, p=0.03) and creatinine and age (r=0.782, p<<0.001) in controls.

*Glycation gap*

From HbA1c-FAM regression (Fig. 3) we obtained the equation for calculation of expected HbA1c values: HbA1c=2.29+3.02xFAM, r=0.601, p<<0.001.

The mean GG of HbA1c expressed in % in diabetic subjects was negative in all diabetic patients (-2.3±14.6 %) and in –DC group (-8.1±11.4 %) and positive in +DC group (2.4±15.4 %) (Fig. 4). The $\chi^2$ test have shown the significant relation of GG of HbA1c to DC occurrence (p=0.0003) (Table 3).

The equation for calculation of expected s-AGEs values was obtained from s-AGEs-HbA1c regression: s-AGEs=2.92+41.64xHbA1c, r=0.443, p<<0.001. The GG of s-AGEs was calculated in %. 15 diabetic subjects –DC (from 35) and 19 diabetic subjects +DC (from 35) were of positive GG, but $\chi^2$ test have not shown relation of GG of s-AGEs to DC occurrence. We have tried to evaluate a relation between GG of s-AGEs and GG of HbA1c in diabetic patients). But no relation were found.

**Discussion**

Vascular complications of diabetes, including retinopathy, neuropathy, nephropathy, and macrovascular disease, are the major cause of morbidity and mortality in diabetic patients, with macrovascular disease being a major cause of premature death. Dysfunction of the key cells responsible for vascular function, including endothelial cells, pericytes, and vascular smooth muscle cells, can be induced by increased cellular concentrations of glucose during hyperglycemia. This can activate multiple pathways of biochemical dysfunction leading to increased glycation of proteins (Ahmed et al. 2005) and to enhanced oxidative and carbonyl stress with subsequent direct tissue damage. According to Galler et al. (2003) HbA1c does not

seem to be directly involved in the development of vascular complications and therefore it would be beneficial to find additional predictors that distinguish between those patients who have a greater risk of developing complications from those who do not. In view of the increasing incidence of childhood diabetes, this would be especially important for subjects with type 1 diabetes whose illness started in childhood.

We have found only a few studies with investigation of clinical, biochemical and glycation parameters of patients with type 1 diabetes with regard to the presence of DC.

In our study TC and HDL levels were significantly higher in all diabetic subjects (regardless to DC presence) than in controls and in –DC group vs. controls. HDL levels were significantly higher also in +DC group vs. controls. Merzouk et al. (2004) observed the similar TC levels in all diabetic groups, TAG and LDL were higher in +DC vs. controls and HDL levels were significantly lower in +DC than those in controls in their article.

The significantly higher levels of FAM were found in all diabetic subjects than in controls in accordance with Ajabnoor et al. (1990), Martinez et al. (1994b), Schalkwijk et al. (1999), Jakuš et al. (2000), and significantly higher FAM levels in diabetic group +DC vs. –DC similarly to Schalkwijk et al. (1999) who presented the higher levels of FAM in diabetic group with nephropathy than without one. Cohen et al. (2008) presented the similar results in patients with and without retinopathy. Martin-Gallan et al. (2003) have not found significant differences in FAM and HbA1c levels of diabetic subjects +DC compared with –DC, but we have found significantly elevated FAM and HbA1c levels in +DC group compared with –DC one.

In accordance with Merzouk et al. (2004) the higher HbA1c levels were observed in both +DC and –DC diabetic group compared with controls.

In accordance with some other studies (Berg et al. 1997b, Chiarelli et al. 2000, Jakuš et al. 2001) authors detected significantly higher s-AGEs levels in diabetic subject on the whole

(regardless of DC presence) vs. controls. In this study the s-AGEs levels in diabetic subjects +DC were significantly higher in comparison with group –DC, but not in –DC group compared with controls. Chiarelli et al. (2000) investigated s-AGEs in patients with nephropathy and retinopathy and with no angiopathies and pretended the severity of diabetic angiopathy is related to s-AGE levels.

In the present study age and duration of disease were associated with creatinine in both diabetic groups, age with HDL (negative correlation) and with TAG in group –DC. These results suggest the eventual association of lipid disorders with complications in relation with age and duration of diabetes. Good management of diabetes seems to be of paramount importance in controlling dyslipidemia.

No correlation was found between FAM and any from lipoproteins in all studied subjects. Many studies presented the significant correlations between HbA1c and lipoproteins levels in serum. According to Ladeia et al. (2006) HbA1c correlated with TC, LDL and TAG including LDL also correlated with duration of diabetes in young diabetic patients. Martinez et al. (1994a) have found significant correlation between HbA1c levels and each of TC, LDL and TAG serum concentrations in diabetic patients. In our study HbA1c was correlated with TAG in subjects +DC. Glycemic control and lipid levels are independently associated in youth (Petitti et al. 2007), but lipid disorders may be present regardless presence of complications in young subjects and may be affected by food.

Some studies provide correlations between FAM and HbA1c in diabetic patients (Baker et al. 1983, 1985a, Koskinen et al. 1987, Ajabnoor et al. 1990, Schalkwijk et al. 1999). In our study there was found significant correlation between FAM and HbA1c in both diabetic groups (+DC, -DC). Baker et al. (1983, 1985b) consider FAM to be a reliable indicator of glycemic control, but Jerntorp et al. (1988) report the value of FAM in clinical practice to be unclear. Ajabnoor et al. (1990) have found poor correlation of FAM with duration of diabetes, but we

did not find any similar relationship. The significant correlation was found between s-AGEs and HbA1c only in diabetic patients +DC.

In our study neither FAM nor HbA1c have correlated with age and duration of disease. S-AGEs were correlated with FAM only in subjects +DC and with HbA1c too. No similar associations were found in –DC subjects.

According to Berg et al. (1997a) and Chiareli et al (2000) the s-AGEs predict the progression of early morphological kidney damage in patients with type 1 diabetes.

Cohen et al. (2003, 2008) investigated GG of HbA1c calculated from FAM in patients with and without nephropathy and with and without retinopathy. The authors present negative GG in subjects without nephropathy or retinopathy and with microalbuminuria and positive GG in subjects with advanced nephropathy and with retinopathy. Similarly, in our study we have found positive GG of HbA1c in +DC and negative in –DC group. Using $\chi^2$ test we also have found the significant relation of GG positivity to occurrence of DC. Cohen et al. (2003, 2008) pretend that the patients with high GG and high risk of nephropathy and retinopathy have a low FAM in relationship to the HbA1c. They demonstrated correlation between GG and nephropathy and retinopathy, but not prediction and they suggest the necessity of prospective studies of this issue. McCarter et al. (2000) think the differences between extracellular and intracellular measures of glycemic control may precede nephropathy and that the hemoglobin glycation index, an analogous measure of discordance between self glucose-monitoring and HbA1c, predicts retinopathy and nephropathy.

There was also calculated GG of s-AGEs predicted from measured actual HbA1c values in our study. 15 diabetic subjects –DC from 35 and 19 diabetic subjects +DC from 35 were of positive GG of s-AGEs. Because hemoglobin is an intracellular protein and FAM and s-AGEs reflect extracellular proteins, the GG could result from differences between the ambient glucose concentrations or rates of glycation in the intracellular and extracellular

compartments or individual differences in the turnover/metabolism of underlying proteins.

We have found surprise high number of positive GG also in –DC group. Unfortunately, despite these findings no relation between GG of s-AGEs and occurrence of DC was proved. The GG of s-AGEs have never been studied. Our idea was to try if GG of s-AGEs could be a predictor of DC development. The GG of s-AGEs does not appear to be a predictor of DC. But we assume this issue requires a longitudinal study.

Because the elevated s-AGEs levels occur not only in subjects with complications, the s-AGEs maybe could predict a development of microangiopathies, but the role of s-AGEs as a marker of later complications development is to be confirmed by long-term studies and the consequent study should be focused on subjects without complications.

We assume s-AGEs appear not only to reflect presence of DC, but also predict the development of them. This issue requires further investigation of glycemic control impact to presence enhanced s-AGEs levels in –DC diabetic subjects.

**Acknowledgements**

Supported by Grant 1/3417/06 from the VEGA Agency. Authors thank MUDr. E. Jančová, MUDr. J. Staník and MUDr. A. Staníková, PhD. for assistance with subject recruitment and assessment.

**References**

AHMED N, BABAEI-JADIDI R, HOWELL SK, THORNALEY PJ, BEISSWENGER PJ: Glycated and oxidized protein degradation products are indicators of fasting and postprandial hyperglycemia in diabetes. *Diabetes Care* **28**: 2465-2471, 2005.

AJABNOOR MA, ZAWAWI TH, MARZOUKI KM, MARZOUKI ZM: Level of serum fructosamine in Saudi diabetic patients. *Acta Diabetol. Lat.* **27**: 105-112, 1990.

BAKER JR, METCALF PA, HOLDAWAY IM, JOHNSON RN: Serum fructosamine concentration as measure of blood glucose control in type I (insulin dependent) diabetes mellitus. *Br. Med. J. (Clin. Res. Ed.)* **290**: 352-355, 1985a.

BAKER JR, O´CONNOR JP, METCALF PA, LAWSON MR, JOHNSON RN: Clinical usefulness of estimation of serum fructosamine concentration as a screening test for diabetes mellitus. *Br. Med. J. (Clin. Res. Ed.)* **287**: 863-867, 1983.

BAKER J, REID I, HOLDAWAY I: Serum fructosamine in patients with diabetes mellitus. *N. Z. Med. J.* **98**: 532-535, 1985b.

BERG TJ, BANGSTAD HJ, TORJESEN PA, OSTERBY R, BUCALA R, HANSSEN KF: Advanced glycation end products in serum predict changes in the kidney morphology of patients with insulin-dependent diabetes mellitus. *Metabolism* **46**: 661-665, 1997a.

BERG TJ, DAHL-JORGENSEN K, TORJESEN PA, HANSSEN KF: Increased serum levels of advanced glycation end products (AGEs) in children and adolescents with IDDM. *Diabetes Care* **20**: 1006-1008, 1997b.

CHIARELLI F, CATINO M, TUMINI S, CIPOLLONE F, MEZZETTI A, VANELLI M, VERROTTI A: Advanced glycation end products in adolescents and young adults with diabetic angiopathy. *Pediatr. Nephrol.* **14**: 841-846, 2000.

CHIARELLI F, VERROTTI A, MOHN A, MORGESE G: The importance of microalbuminuria as an indicator of incipient diabetic nephropathy: therapeutic implications. *Ann. Med.* **29**: 439-445, 1997.

COHEN MP, ZIYADEH FN, HONG SW, SHERMAN CW, HUD E, LAUTENSLANGER GT, IGLESIAS-DE LA CRUZ MC, CHEN S: Inhibiting albumin glycation in vivo ameliorates glomerular overexpression of TGF-β1. Kidney Int. **61**: 2025-2032, 2002.

COHEN RM, HOLMES YR, CHENIER TC, JOINER CH: Discordance between HbA1c and fructosamine: evidence for a glycosylation gap and its relation to diabetic nephropathy. *Diabetes Care* **26:** 163-167, 2003.

COHEN RM, LE CAIRE TJ, LINDSELL CJ, SMITH EP, D´ALESSIO DJ: Relationship of prospective GHb to glycated serum proteins in incident diabetic retinopathy: implications of the glycation gap for mechanism of risk prediction. *Diabetes Care* **31**: 151-153, 2008.

GALLER A, MÜLLER G, SCHINZEL R, KRATZSCH J, KIESS W, MÜNCH G: Impact of metabolic control and serum lipids on the concentration of advanced glycation end products in the serum of children and adolescents with type 1 diabetes, as determined by fluorescence spectroscopy and *N*ε-(Carboxymethyl)Lysine ELISA. *Diabetes Care* **26**: 2609-2615, 2003.

GUGLIUCCI A: Glycation as the glucose link to diabetic complications. *J. Am. Osteopath. Assoc.* **100:** 621-634, 2000.

HANSSEN KF: Blood glucose control and microvascular and macrovascular complications in diabetes. *Diabetes* **46** (Suppl. 2): S101-S103, 1997.

JAKUŠ V, BAUEROVÁ K, MICHALKOVÁ D, ČÁRSKY J: Values of markers of early and advanced glycation and lipoxidation in serum proteins of children with diabetes mellitus. *Bratisl. Lek. Listy* **101:** 484-489, 2000.

JAKUŠ V, BAUEROVÁ K, MICHALKOVÁ D, ČÁRSKY J: Serum levels of advanced glycation end products in poorly metabolically controlled children with diabetes mellitus: relation to HbA1c. *Diabetes Nutr. Metab.* **14**: 207-211, 2001.

JAKUŠ V, RIETBROCK N: Advanced glycation end-products and the progress of diabetic vascular complications. *Physiol. Res*. **53**: 131-142, 2004.

JANUSZEWSKI AS, THOMAS MC, KARSCHIMKUS CS, CHUNG JS, ROWLEY KG, NELSON CL, O´NEAL DN, DRAGICEVIC G, HARPER CA, BEST JD, JENKINS AJ: Longitudinal analysis of low-molecular weight fluorophores in type 1 diabetes mellitus. *J. Med. Invest.* **55**: 29-36, 2008.

JERNTORP P, SUNDKVIST G, FEX G, JEPPSSON JO: Clinical utility of serum fructosamine in diabetes mellitus compared with hemoglobin A1c. *Clin. Chim. Acta* **175:** 135-142, 1988.

JOHN WG, MOSCA A, WEYCAMP C, GOODALL I: $HbA_{1c}$ Standardisation: History, Science and Politics. *Clin. Biochem. Rev.* **28**: 163–168, 2007.

KOSKINEN P, IRJALA K, VIIKARI J, PANULA-ONTTO R, MATIKAINEN MT: Serum fructosamine in the assessment of glycaemic control in diabetes mellitus. *Scand. J. Clin. Lab. Invest.* **47**: 285-292, 1987.

KUMAR MS, REDDY PY, KUMAR PA, SUROLIA I, REDDY GB: Effect of dicarbonyl-induced browning on α-crystallin chaperone-like activity: physiological significance and caveats of *in vitro* aggregation assays. *Biochem. J.* **379**: 273-282, 2004.

LADEIA AM, ADAN L, COUTO-SILVA AC, HILTNER A, GUIMARÃES AC: Lipid profile correlates with glycemic control in young patients with type 1 diabetes mellitus. *Prev. Cardiol. Spring* **9:** 82-88, 2006.

LITTLE RR, GOLDSTEIN DE: Endocrine (standardization of glycohemoglobin measurement). *Anal. Chem.* **67**: 393R-397R, 1995.

MARTÍN-GALLÁN P, CARRASCOSA A, GUSSINYÉ M, DOMÍNGUEZ C: Biomarkers of diabetes-associated oxidative stress and antioxidant status in young diabetic patients with or without subclinical complications. *Free Radic. Biol. Med.* **34:** 1563-1574, 2003.

MARTINEZ MT, RAMOS O, CARRETERO N, CALVILLAN M, GUTIERREZ-LÓPEZ MD, CUESTA P, SERRANO-RIOS M: Lipoprotein (a) and other risk factors in children with insulin-dependent diabetes mellitus and children without diabetes. *Diabete Metab*. **20**: 522-525, 1994a.

MARTINEZ MT, PÉREZ F, CAVILLÁN M, GUTIERREZ-LÓPEZ MD, SERRANO-RIOS M (1994b): Lipoprotein (a) and other risk factors in Chilean children with type I diabetes mellitus. *Rev. Med. Chil.* **122**: 1115-1119, 1994b.

MC CARTER R, HEMPE J, GOMEZ R, CHALEW SA: Hemoglobin glycosylation index is a predictor of complications in the DCCT (Abstract). *Diabetes* **49** (Suppl. 1): A48, 2000.

MERZOUK S, HICHAMI A, SARI A, MADANI S, MERZOUK H, YAHIA BERROUIGUET A, LENOIR-ROUSSEAUX JJ, CABANE-SARI N, KHAN NA: Impaired oxidant/antioxidant status and LDL-fatty acid composition are associated with increased susceptibility to peroxidation of LDL in diabetic patients. *Gen. Physiol. Biophys.* **23**: 387-399, 2004.

MICHALKOVÁ D, ČÁRSKY J, BIRČÁK J, KOLÁŘ J, BAČIAKOVÁ Ľ, VITKOVÁ M: Glycosylated Hemoglobin HbA1c as Indicator of Metabolic Compensation in Children Suffering from Diabetes Mellitus. *Čs. Pediatr.* **36**: 243-247, 1981 (in Slovak).

MOGENSEN CE: Should ACE inhibitors be used in normotensive micro-albuminuric IDDM? *Diabetes Rev. Int*. **3**: 2-6, 1995.

MOJTO V., TISOŇ P.: Principles of prevention and therapy in diabetic nephropathy. *Bratisl. Lek. Listy* **105**: 432-433, 2004.

PETITTI DB, IMPERATORE G, PALLA SL, DANIELS SR, DOLAN LM, KERSHNAR AK, MARCOVINA S, PETTITT DJ, PIHOKJER C: Serum lipids and glucose control: the SEARCH for Diabetes in Youth study. *Arch. Pediatr. Adolesc. Med.* **161**: 159-165, 2007.

RÁCZ O, VÍCHA T, PAČIN J: Glycohemoglobin, Glycation of Proteins and Diabetes Mellitus. Osveta, Martin, 1989 (in Slovak).

RÁCZ O, ŠIPULOVÁ A: Microangiopathy. In: Diabetes Mellitus. J Vozár, A Kreze, Klimeš (eds.), Slovak Academic Press, Bratislava, 1998, pp. 171-177 (in Slovak).

SAMPATHKUMAR R, BALASUBRAMANYAM M, REMA M, PREMANAND C, MOHAN V: A novel advanced glycation index and its association with diabetes and microangiopathy. *Metabolism* **54**: 1002-1007, 2005.

SCHALKWIJK CG, LIGTVOET N, TWAALFHOVEN H, JAGER A, BLAAUWGEERS HGT, SCHLINGEMANN RO, TARNOW L, PARVING H-H, STEHOUWER CDA, VAN HINSBERGH VWM: Amadori albumin in type 1 diabetic patients: correlation with markers of endothelial function, association with diabetic nephropathy, and localization in retinal capillaries. *Diabetes* **48**: 2446-2453, 1999.

THORPE SR, BAYNES JW: Maillard reaction products in tissue proteins: new products and new perspectives. *Amino Acids* **25**: 275-281, 2003.

WAUTIER JL, SCHMIDT AM: Protein glycation: a firm link to endothelial cell dysfunction. *Circ. Res.* **95**: 233-238, 2004.

Table 1. Clinical and biochemical parameters in diabetic patients and controls

| | Children and adolescents with DM1 | n | Children and adolescents DM1 -DC | n | Children and adolescents DM1 +DC | n | Controls | n |
|---|---|---|---|---|---|---|---|---|
| Age (years) | 15.06±2.66 | 76 | 14.19±3.17 | 35 | 15.81±1.81 | 41 | 9.25±4.85 | 30 |
| Diabetes duration (years) | 8,68±3,02 | 76 | 7,54±2,51[2] | 35 | 9,64±3,08[1] | 41 | | |
| HbA1c (%) | 9.53±1.88[3] | 76 | 8.41±1.19[2,3] | 35 | 10.48±1.83[1,3] | 41 | 5.0±0.38 | 20 |
| FAM (mmol/l) | 2.850±0.495[3] | 75 | 2.614±0.430[2,3] | 34 | 3.043±0,459[1,3] | 41 | 1.62±0.34 | 29 |
| s-AGEs (U.A.) | 69.4±12.4[3] | 70 | 65.8±9.05[2] | 35 | 73.0±14.09[1,3] | 35 | 60.17±13.78 | 30 |
| TC (mmol/l) | 4.24±0.77[3] | 76 | 4.3±0.69[3] | 35 | 4.19±0.83 | 41 | 3.91±0.72 | 30 |
| HDL (mmol/l) | 1.65±0.59[3] | 76 | 1.72±0.45[3] | 35 | 1.58±0.67[3] | 41 | 1.29±0.32 | 30 |
| LDL (mmol/l) | 2.60±0.67 | 76 | 2.53±0.68 | 35 | 2.66±0.65 | 41 | 2.43±0.64 | 30 |
| TAG (mmol/l) | 1.20±0.86 | 76 | 0.90±0.47[2] | 35 | 1.45±1.02[1] | 41 | 1.37±0.67 | 29 |
| Creatinine (μmol/l) | 58.52±11.27[3] | 75 | 57.09±11.04[3] | 34 | 59.1±11.33[3] | 41 | 42.43±12.25 | 28 |
| UAER (μg/min) | 38.88±119.69 | 71 | 7.95±4.03[2] | 35 | 67.34±160.98[1] | 36 | | |

Data are presented as means±SD.

[1]- significantly different compared with diabetic group -DC

[2]- significantly different compared with diabetic group +DC

[3]- significantly different compared with controls

Table 2. Correlations between clinical and biochemical parameters of diabetic subjects

| Group | | HbA1c - FAM | HbA1c - s-AGEs | FAM-s-AGEs | HbA1c -TAG | Age - HDL | Age - TAG | Age - creatinine | Duration of DM - creatinine |
|---|---|---|---|---|---|---|---|---|---|
| +DC | r | 0.374 | 0.478[1] 0.366[2] | 0.396 | 0.433 | | | 0.542 | 0.452 |
| | p | 0.002 | 0.003[1] 0.03[2] | 0.02 | 0.005 | >0.05 | >0.05 | <<0.001 | 0.003 |
| | n | 41 | 35 | 35 | 41 | | | 41 | 41 |
| -DC | r | 0.719 | | | | -0.386 | 0.352 | 0.684 | 0.407 |
| | p | <<0.001 | >0.05 | >0.05 | >0.05 | 0.02 | 0.04 | <<0.001 | 0.02 |
| | n | 34 | | | | 35 | 35 | 34 | 34 |

[1]mean HbA1c values in previous 2 years

[2]actual HbA1c values

Table 3. Occurrence of diabetic complications in relation to GG of HbA1c

| Occurrence of DC | +DC | -DC | Sum |
|---|---|---|---|
| Number of subjects with positive GG of HbA1c | 26 | 7 | 33 |
| Number of subjects with negative GG of HbA1c | 15 | 27 | 42 |
| Sum | 41 | 34 | 75 |

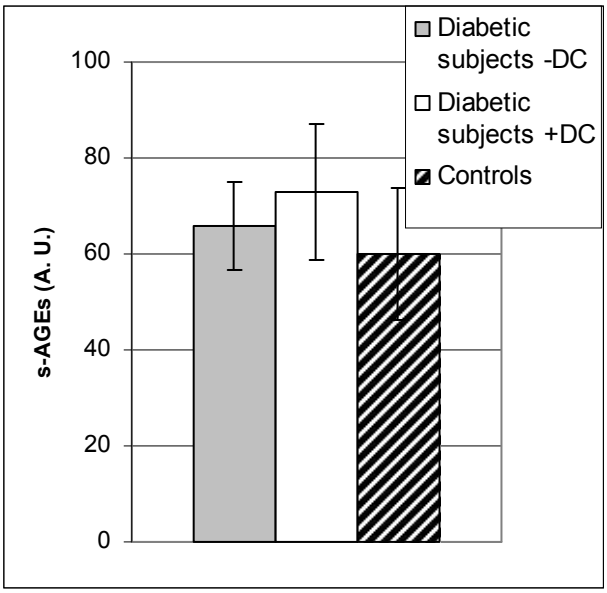


Fig. 1. Comparison of s-AGEs levels of diabetic subjects and controls ($p_1 << 0.001$, $p_2 > 0.05$, $p_3 < 0.05$, $p_1$: +DC vs. controls, $p_2$: -DC vs. controls, $p_3$: +DC vs. –DC)

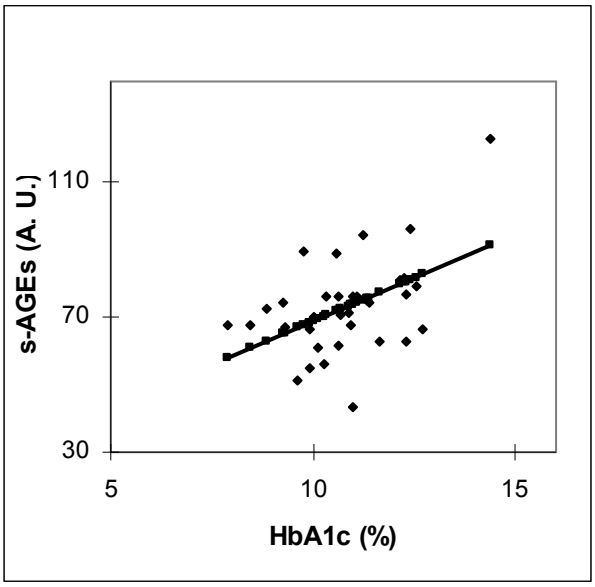


Fig. 2. Correlation between s-AGEs and HbA1c (mean of the last 2 years) in diabetic subjects with complications ($r = 0.478$, $p < 0.01$)

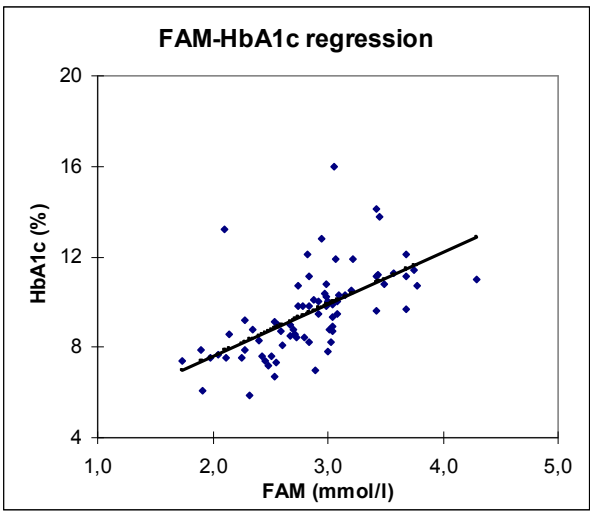


Fig. 3. FAM-HbA1c regression

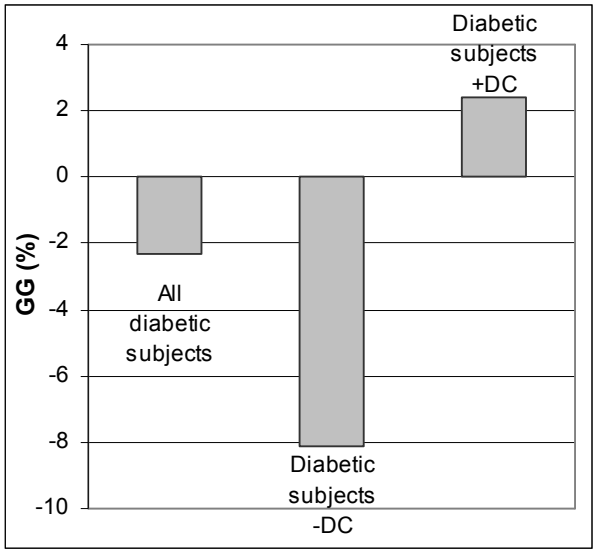


Fig. 4. Mean GG of HbA1c (in %) calculated from FAM in diabetic subjects