Glycative Stress Research

Online edition : ISSN 2188-3610
Print edition : ISSN 2188-3602
Received: October 10, 2015
Accepted : January 6, 2016
Published online : March 31, 2016

Original Article

Relation between glycemia level and common carotid artery intima-media thickness in females

Nikola Barić [1)], Sanja Barić Santro [2)]

1) Private General Practice Office, Labin, Croatia
2) Departament for Pediatry, Istrian Health Centers-Branch Unit Dr. Lino Peršić Labin, Croatia

Abstract

***Objective*:** The onset and course of carotid atherosclerosis are strongly influenced by interconnected effects of well-known risk factors, each of which influence this disease. Using standard laboratory biochemical methods and high resolution ultrasound technics, we aimed to determine the manner by which different fasting glycemia levels influence the onset of the first signs of atherogenesis on carotid artery walls. The value of intima-media complex thickness (CCA-IMT) of the common carotid artery on both sides of the neck was used as it is a marker of such changes.

***Methods*:** A total of 97 conditionally healthy women aged 17-92, average age 53.42 ± 16.59 years, made up the study subjects. The correlation between the fasting glycemia level and the values of the carotid artery intima-media complex was investigated. Morning glycemia values were determined for each examinee by standard biochemical analysis; using high resolution ultrasound, bilateral values of intima-media thickness were measured on the longitudinal image of the common carotid artery cross section. The ultrasound color Doppler device SA 6000C (Kretztechnik A.G., Austria) with a linear transducer of 7.5 MHz was used. Statistical analysis of the obtained results was made by the univariate linear regression analysis method.

***Results*:** Obtained results indicate the well noticeable linear positive connection between the fasting glycemia level and intima-media thickness wall of both common carotid arteries. On the left side the connection was statistically significant: Pearson coefficient $r = 0.264$, $p = 0.009$, $p < 0.05$; on the right side the connection was positive but not statistically significant; $r = 0.184$, $p = 0.071$, $p > 0.05$. Confidence (CI) on both sides was 95%.

***Conclusion*:** The fasting glycemia level showed strong positive correlation with the common carotid artery intima-media thickness complex. As this parameter represents a strong marker of already existing or a possible future carotid and generalised atherosclerosis, the importance of glycemia regulation is evident, especially in persons with Type 1 or Type 2 diabetes.

***KEY WORDS*:** fasting glycemia level, glycative stress, common carotid artery intima-media thickness, carotid atherosclerosis

Introduction

Well-known harmful effects of hyperglycemia on the onset and course of carotid atherosclerosis are the motive for a series of investigations on the etiology of these effects. Primary causes of hyperglycemia and its harmful effects are both types of diabetes mellitus (Types 1 and 2) which have a crucial role in its pathophysiology. Both types, according to recent investigations, are caused by interractions between genetic factors and certain environmental factors. The precise cause is yet unknown. Therefore, this investigation was undertaken to analyse the influence of different levels of glycemia (in the range of euglycemia, 80-110 mg/dL, 4-6 mmol/L) on the common carotid artery wall and the appearance of the first signs of alterations, although barely visible, but potentially dangerous as a reason for future localised and generalised atherosclerosis. Thus, this investigation focused on the actual analysis of subtle alterations in the arterial wall induced by variations in euglycemia among conditionally healthy women [1-4)].

Carotid atherosclerosis, which is increasing in incidence and prevalence, is the crucial factor in the appearance of thromboembolism of cerebral arteries with ischemic brain stroke; the painful consequences are often chronic and

Address correspondence to: Barić Nikola MD, PhD
Private General Practice Office Labin
Presika 153, 52220 Labin, Istra, Croatia
Tel. 385/052/852-008/ E-mail: nikola.baric@pu.t-com.hr
Co-author: Sanja Barić Santro
Department for pediatry, Istrian Health Centers-Branch Unit Dr. Lino Peršić Labin
Svetog Mikule 2, 52220, Labin, Istra, Croatia
Tel. 385/052/855-333/ inter.121/ E-mail: sanja.baricsantro@gmail.com

lethal complications. Death from brain stroke holds a high position among the causes of death in modern societies. In order to put a stop to this dangerous course, there is critical need for the strong prevention of carotid atherosclerosis, indicating the necessity for quick and simple diagnostics of the first signs. For this reason, it is necessary to undertake ultrasonic investigation of all three carotid artery walls and measurement of the common carotid artery intima-media thickness (CCA-IMT) which has recently been recognised as a possible powerful marker of wall damages. This is the optimal method to investigate the abberations from normal values in accordance with age. This method indicates the great possibility for the detection of actual or the risk of future atherosclerosis. High resolution carotid artery ultrasonic investigation is a quick, exact and cost effective method to solve the problem [5].

Normal values of the fasting blood glucose test in adult persons are between 80-110 mg/dL, (4-6 mmol/L) for normoglycemia; values over 126 mg/dL (> 7 mmol/L) for hyperglycemia, and values below 70 mg/dL (< 4 mmol/L) for hypoglycemia. The present study is primarily involved in the analysis of mild subtle differences in the blood glucose level in the range of normoglycemia. The regression lines presented indicate that differences exist (see ***Fig. 7***). Hyperglycemia also occurs in some other diseases and pathologic conditions.

The mechanism by which hyperglycemia renders damages to the arterial wall has recently been investigated in detail by the analysis of the process of nonenzymatic glycosilation or glycation (Maillard reaction, browning reaction) of proteins and lipids and the obtained compounds, the so-called "advanced glycation end products" (AGEs). HbA1c (glycosylated hemoglobin) is one of these compounds. The deteriorative effects of these compounds are recently under intensive investigations (***Figs. 1-3***) [1,3,6-12].

Numerous investigations emphasise that elevated glucose metabolism accompanied by hyperglycemia induces the rise in the generation of reactive oxygen species (ROS) in mitochondria and (NAD(P)H) oxidase activation in cytoplasm. NAD(P)H oxidase generates superoxide anion O_2^{*-} (a ROS). By direct deteriorative effects on cellular structures (especially lipid membranes) or as signaling molecules, these compounds (ROS) activate signal pathways for the transcription of various proteins with atherogenic activity. The IKK-β/NF-κB pathway [subunit of kappa B kinase (IKK)/Nuclear factor kappa B] is well studied. Deteriorative changes on arteries observed in diabetes and accompanied by hyperglycemia, are also detected during normal, physiological aging (***Fig. 4***).

Methods

The study was performed from 2008 to 2013. The women studied included 97 carefully selected examinees according to the specific criteria with the aim to closely correspond to the supposed values of physiological or natural ageing. The age of examinees ranged from 17 to 92 years-average age was 53.42 ± 16.59 years. At the beginning of the investigation, 173 female patients, who visited our institution for a variety of disorders, primarily cervical, cervicobrachial syndrome, headache, vertigo and instability, and for standard neurological and CD/TCD examination, were examined. After detailed examination of the extracranial and intracranial arterial systems, persons without manifested carotid atherosclerosis were explained about the importance of CCA-IMT measuring and the fact that their results would be very useful for them and for the actual investigation. The privacy of the subjects regarding the obtained results was guaranteed. Before inclusion in the study, consent was obtained from every examinee. The investigation was performed in accordance with general ethical norms and laws.

Glucose values were determined according to the standard procedures in the biochemical laboratory in the Health Center Labin. Fasting glucose (in mg/dL and mmol/L) was determined by the photometric method with oxidase. Referential range was 4.40-6.40 mmol/L (80-110 mg/dL). Examinees with glucose values of over 9.5 mmol/L (171 mg/dL) were not included in the study. We arbitrarily took this value which was slightly below the renal threshold of 180 mg/dL for glucosuria. The analysis included the mean fasting glucose value from at least two of the last registered measurements. For practical reasons, in order to determine the individual difference between the fasting glucose value of all examinees and the standard value typical for age, we compared our obtained mean glycemic values with values on the regression line CCA-IMT/age as previously reported [5]. However, the methodology used has some limitations. The individual HbA1c value measurement and glucose tolerance test (determination of possible postprandial hyperglycemia) were not performed. The reason for this approach was based on our opinion that we were interested in nondiabetic examinees. We decided only to analyse the relation between fasting plasma glucose (FPG) and CCA-IMT.

The measurement of CCA-IMT was performed on the longitudinal CCA cross-section on its distal part, at 1-1.5 cm proximal to the bulb border. Measurement was performed in the terminal phase of diastole on the arterial far wall, which is superior to near wall measurements. Transducer position was lateral. The CCA-IMT (mean value of three successive measurements) value was determined by the distance between the leading edge of the lumen/intima border and the leading edge of the media/adventitia border. Values of IMT were presented in mm (***Fig. 5***) [5]. The investigation was carried out by the use of the color duplex doppler device SA 6000C (Kretztechnik A.G., Austria) with the linear transducer of 7.5 MHz (high-resolution B-mode carotid ultrasonography). Examination was performed in the standard supine position. The measuring of CCA-IMT was performed by standard mechanic visual measuring. We think such type of measuring is satisfactory if performed by an experienced operator (***Fig. 6***) [5]. The difference compared with automatic measuring is minimal. After three successive measurements, the mean value was taken as the final result. Obtained IMT values were correlated with adequate glycemia (normoglycemia) values. In this investigation, the univariate linear regression analysis was used. A particular original non-licensed computer program for univariate linear regression analysis was developed by a computer expert from the Holcim Company, following the analysis of standard statistic literature. Our computer program for determining the linear regression was compared with other licensed programs and the results were identical.

After detailed consideration, we concluded that this investigation did not yet have indications to perform multivariate analysis. However, our plan is to undertake this analysis in the near future (***Tables 1-3***). The obtained results are presented graphically (***Fig. 7***) [13].

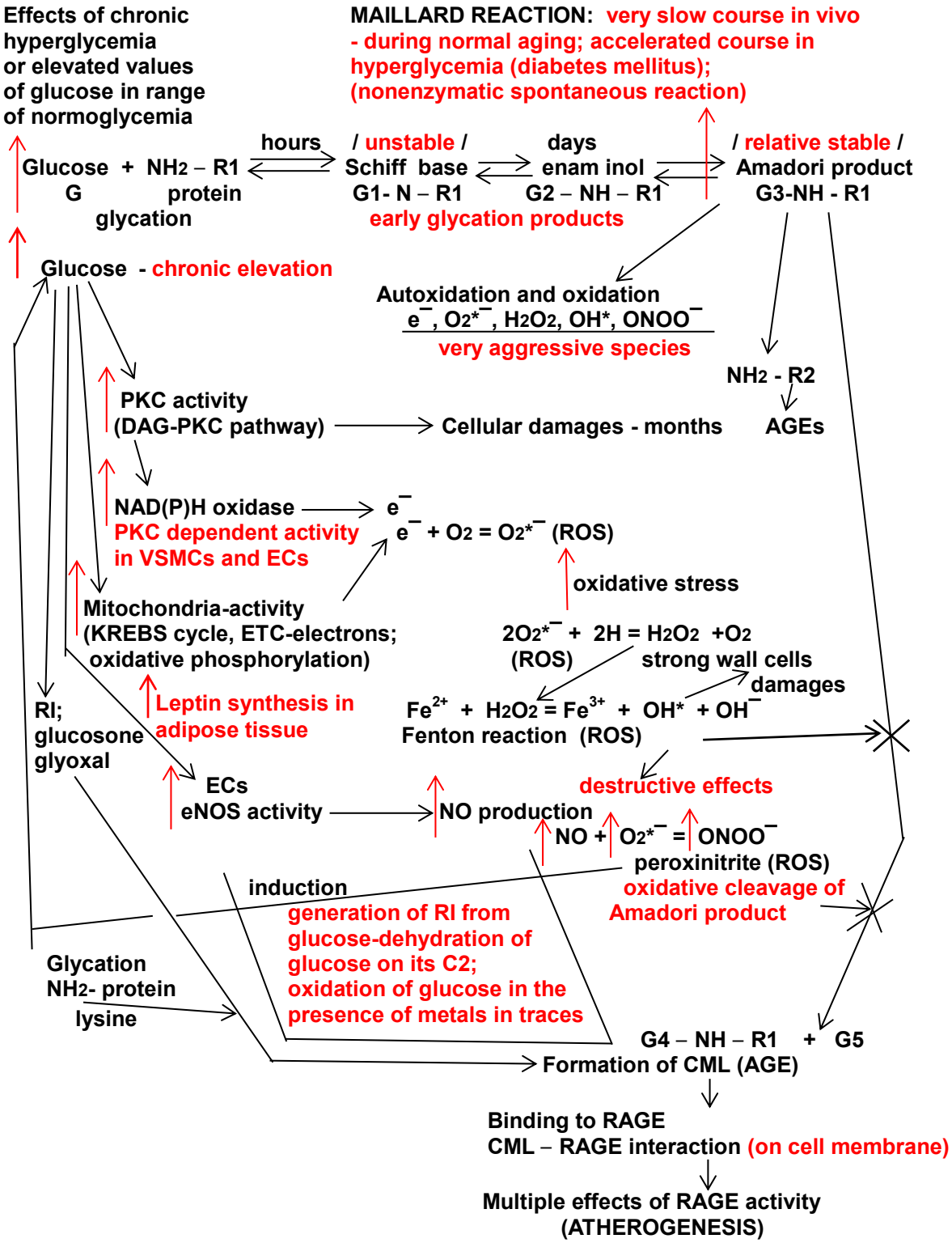

Fig. 1. **Molecular mechanisms of the destructive effects of elevated values of glucose**

AGEs, advanced glycation end products; RAGE, receptor for AGE; RI, reactive intermediates; O2*–, superoxide anion radical – very aggressive; G,glucose; PKC, protein kinase C; NAD(P)H, nicotinamide adenine dinucleotide phosphate oxidase; DAG, diacyl-glycerol; VSMCs, vascular smooth muscle cells; Ecs, endothelial cells; ROS, reactive oxygen species; Leptin, pro-atherogenic adipokine produced by adipose tissue; -NH2, amino group; ETC, electron transport chain; CML, N^{ε}-(carboxymethyl)-lysine; NO, nitric oxide; eNOS, endothelial NO synthase.

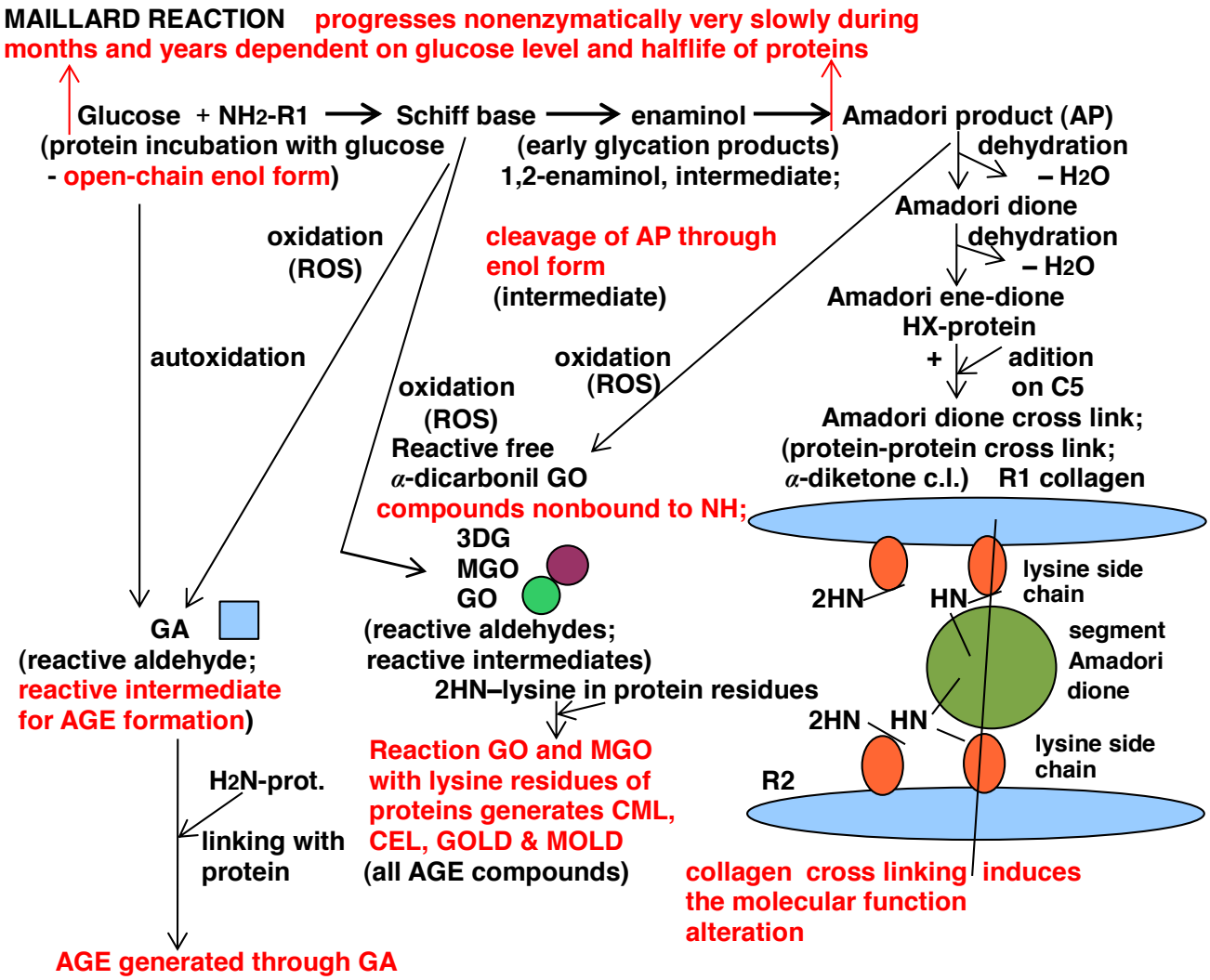

Fig. 2. **Presentation of some aspects of AGE formation and their action**

CL, cross link; CEL, N^{ε}-(carboxyethyl)-lysine; CML, N^{ε}-(carboxymethyl)-lysine; GOLD, glyoxal-lysine dimer; MOLD, methylglyoxal-lysine dimer; AGEs, advanced glycation end products; GOLD and MOLD are AGE-compounds; the great importance of ROS in AGEs generation is evident; -NH_2, amino group; R, long chain of amino acid residues; CEL and CML, AGE compounds; AP, Amadori product; ROS, reactive oxygen species; 3DG, 3-deoxyglucosone; GO, glyoxal; MGO, merthyglyoxal; GA, glycolaldehyde.

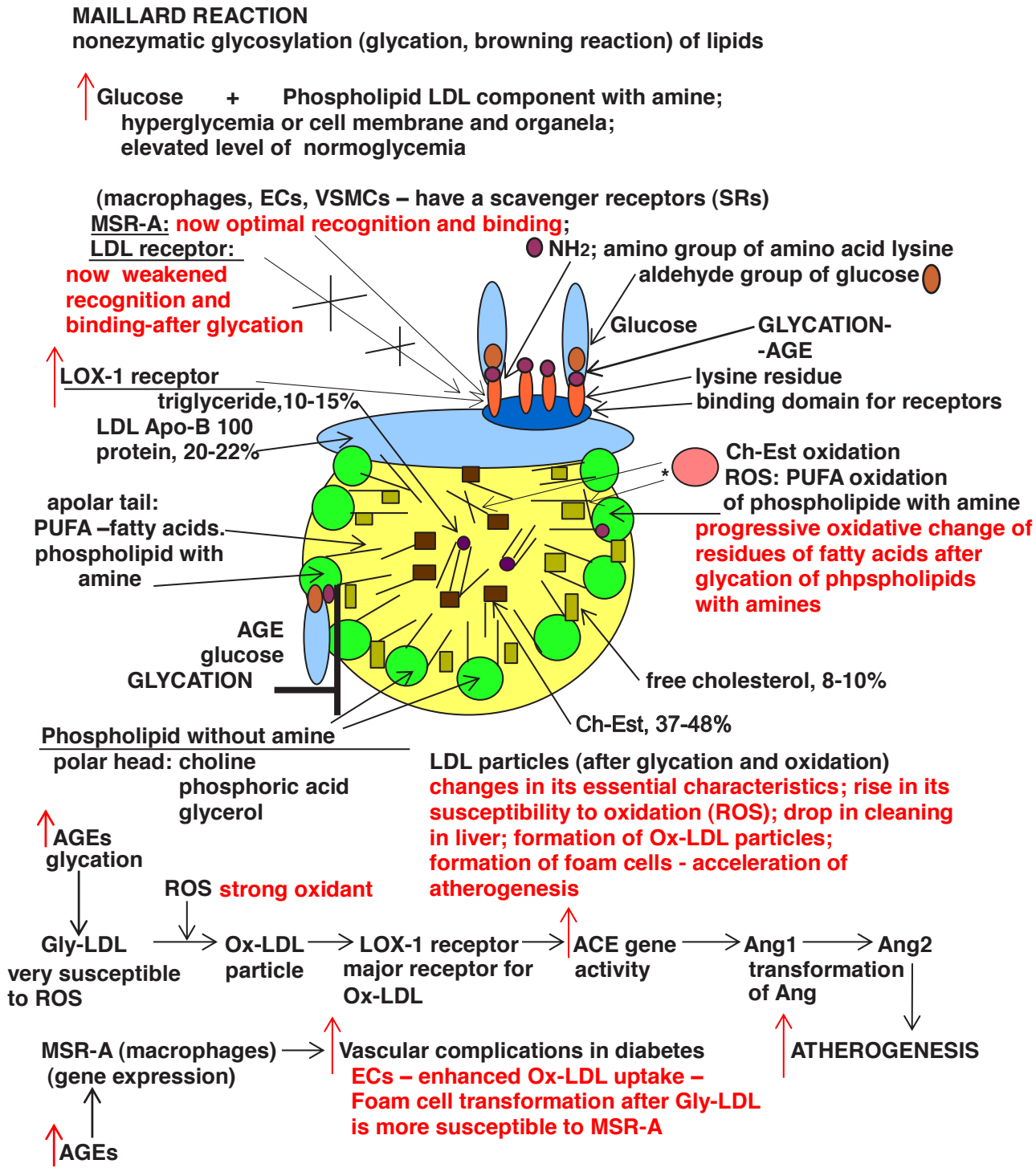

Fig. 3. **Presentation of LDL particle glycation, formation of Ox-LDL, activation of ACE gene, formation of Ang2 and atherogenesis acceleration**

LDL glycosylation (glycation) increases its atherogenicity; LOX-1, receptor for Ox-LDL is located in endothelial cells; MSR-A, macrophage scavenger receptor; PUFA, poliunsaturate fatty acid; LDL receptor is abundant on liver cells. It picks up LDL particles from the blood stream, transports them into the cells with their disintegration. MSR-A (Types 1 and 2) elevates uptake and intracellular deposition of glycated LDL and accelerates atherogenesis; phospholipids in LDL total, with and without amine, is 20-28%; ACE angiotensin converting enzyme; LDL, low density lipoprotein; Ox-LDL, oxidized LDL; Ang2, angiotensin 2; Ch Est, cholesterol ester; VSMCs, vascular smooth muscle cells.

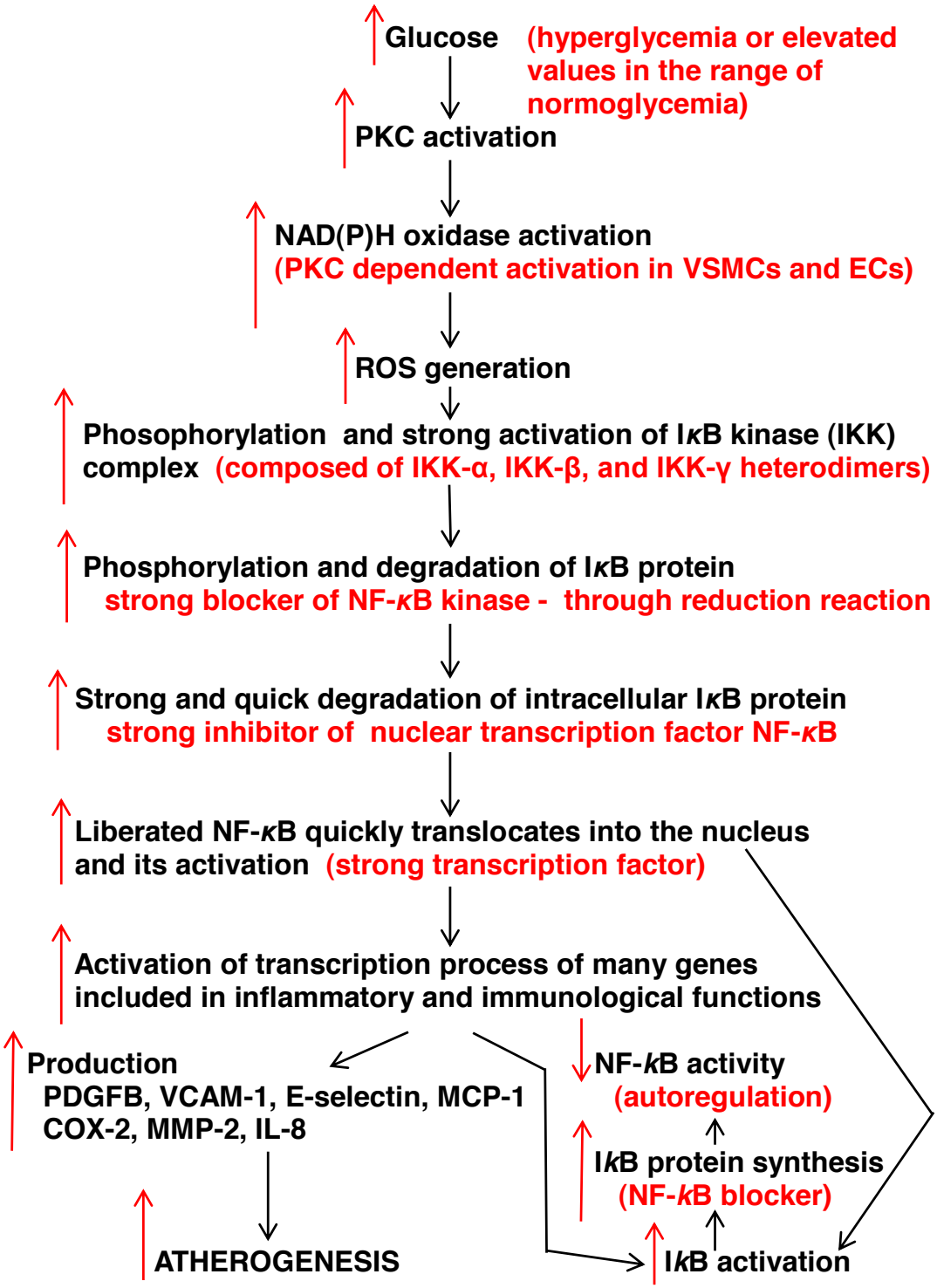

Fig. 4. **A possible pathway for hyperglycemia-induced atherogenesis**

NAD(P)H, nicotinamide adenine dinucleotide phosphate oxidase;NF-κB,nuclear factor kappaB; IκB kinase (IKK) complex, enzyme complex – a part of the NF-κB signal transduction cascade; ECs,endothelial cells; PDGFB, platelet-derived growth factor beta; VCAM-1, vascular cell adhesive molecule-1; E-selectin, endothelial leukocyte adhesion molecule-1; MCP-1, monocyte chemoattractantprotein-1; COX-2, PTGS-2, cyclooxigenase-2; MMP-2, matrix metallopeptidase-2; IL-8, interleukin-8; ICAM-1, intercellular adhesion molecule-1; PKC,protein kinase C; VSMCs; vascular smooth muscle cells; Ecs, endothelial cells; ROS, reactive oxygen species.

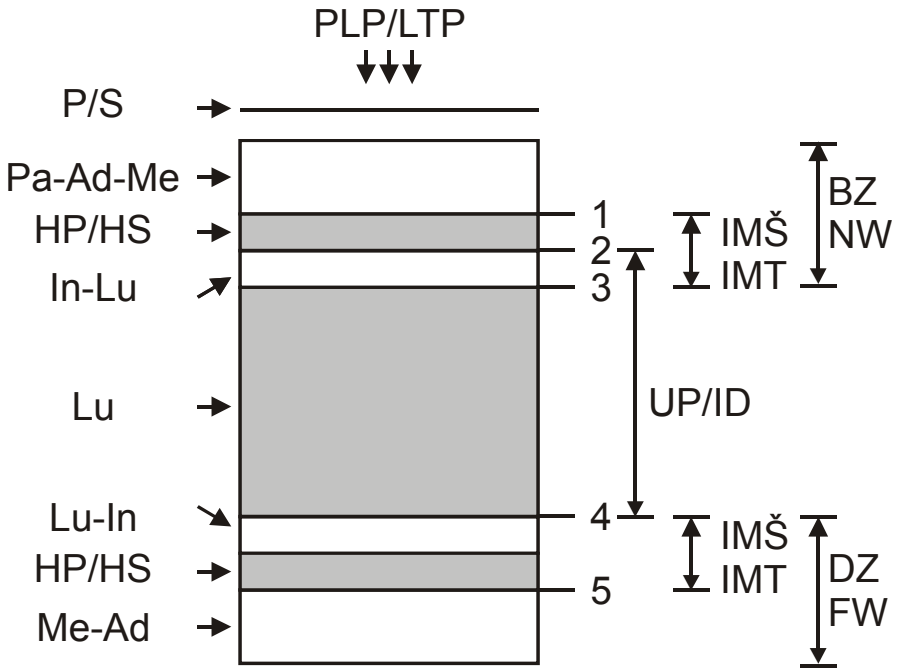

Fig. 5. **Scheme of the longitudinal common carotid artery cross section.**

PLP/LTP = position of linear transducer (7.5 MHz); P/S = skin surface; Pa- Ad-Me = border between periadventitia, adventitia and media; HP = hypoechogenic space; In-Lu = border between intima and lumen; Lu = common carotid artery lumen; 1 = back edge of the periadventitia-adventitia-media border – near wall; 2 = leading edge of the intima-lume border – near wall; 3 = back edge of the intima-lumen border – near wall; 4 = leading edge of the lumen-intima border – far wall; 5 = leading edge of the border media-adventitia – far wall; IMŠ/IMT = intima-media thickness; UP/ID = internal diameter; BZ/NW, near wall; DZ/FW = far wall.

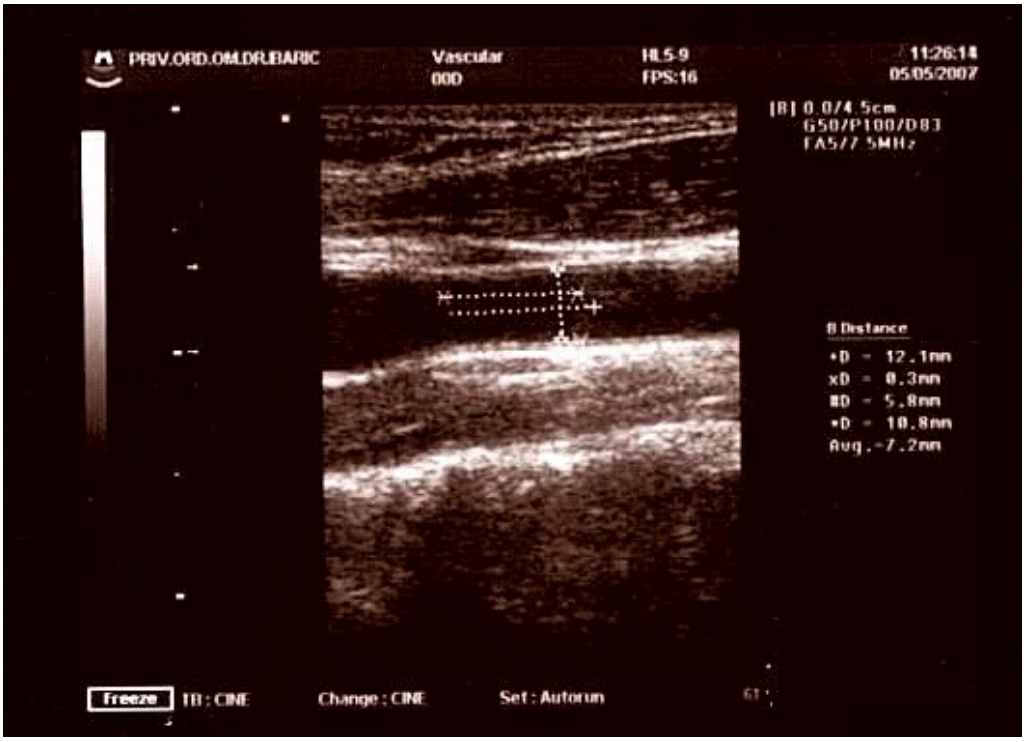

Fig. 6. **Longitudinal US B-mode image of the right CCA in a 24-year-old woman.**

The inside diameter (ID) was 5.8 mm, and the intima-media complex thickness (IMT) was 0.3 mm; Measurement was performed at about 1 cm proximal to the bulb border; lateral position of the linear transducer (7.5 MHz); US, ultrasound, CCA, common carotid artery.

Table 1. Basic characteristics of the women examined.

Characteristic (parameter)	Value	
Total number of examinees (N)	97	
Mean age (years)	53.41 ± 16.59	
Age range (years)	17 – 92	
		N
CCA-IMT < 1.1 mm (%)	100.00	97
CCA-IMT r (mm)	0.455 ± 0.135	
CCA-IMT l	0.472 ± 0,143	
CCA-ID r	6.074 ± 0,608	
CCA-ID l	6.041 ± 0,609	
	%	N
Normal heart rhythm	98.96	96
Mobility	100.00	97
Never smoker	80.41	78
Past smoker	4.12	4
Current smoker	15.46	15
Alcoholics	0	0
Previous (old) stroke	1.03	1
Previous (old) myocardial infarction	1.03	1
	%	N
ICA without stenosis (0%):	88.66	86
ICA - mild stenosis (0-15%):	9.28	9
ICA - moderate stenosis (15-50%)	2.06	2
ICA - strong stenosis (50-70%):	0	0
ICA - critical stenosis (> 70%)	0	0
ICA - occlusion (100%)	0	0
Hemodynamics in order	100.00	97
	%	N
Diabetes Type I (insulin dependent)	0	0
Diabetes Type II (insulin independent)	4.12	4

Abbreviations: CCA-IMT = common carotid artery-intima media thickness-far wall; r = right; l = left; CCA-ID = common carotid artery-inner diameter; Ultrasonographic criteria for quantification ICA stenosis - according to Robinson (Robinson ML, et al. Am Journal Roentgenol. 1988;151:1045); all CCA-IMT are below 1.1 mm; N = total number of examinees; n = number of examinees with a certain characteristic.

Table 2. Basic characteristics of the examined women.

Characteristic (parameter)	Value
Total number of examinees (N)	97
Age (years)	53.41 ± 16.59
Age range (years)	17 – 92
Fasting blood sugar (mmol/L)	5.15 ± 0.77
Elevated blood sugar (n [%])	4 [4.12]
Total cholesterol (mmol/L)	5.48 ± 1.23
Elevated cholesterol (n [%])	49 [50.51]
Triglycerides (mmol/L)	1.17 ± 0.59
Elevated triglycerides (n [%])	11 [11.34]
Height (cm)	163.09 ± 6.20
Weight (kg)	65.96 ± 9.54
BMI (kg/m^2)	24.82 ± 3.42
Systolic pressure (mmHg)	125.38 ± 17.4
Diastolic pressure (mmHg)	79.43 ± 9.54
Pulse pressure (mmHg)	45.95 ± 12.29
Receiving anti-hypertensive medication (n [%])	19 [19.59]

Elevated values are all below the border values for exclusion. Values are presented numerically and in percentages. N; Total number of examinees (N = 97); n, number of examinees with a certain characteristic.

Table 3. Excluded characteristic values.

Characteristic (parameter)	Border values for exclusion
Body mass index (BMI)	> 33.5 kg/m^2
Systolic blood pressure	> 180 mmHg
Diastolic blood pressure	> 110 mmHg
Fasting glucose	> 9.5 mmol/L
Total cholesterol	> 8.7 mmol/L
Triglycerides	> 3.5 mmol/L
Smokers	> 20 cigarettes/day
Insulin-dependent diabetics	Excluded
Verified alcoholics	Excluded
Immobility	Excluded

Total number of examinees (N = 97); See explanation in the Methods section.

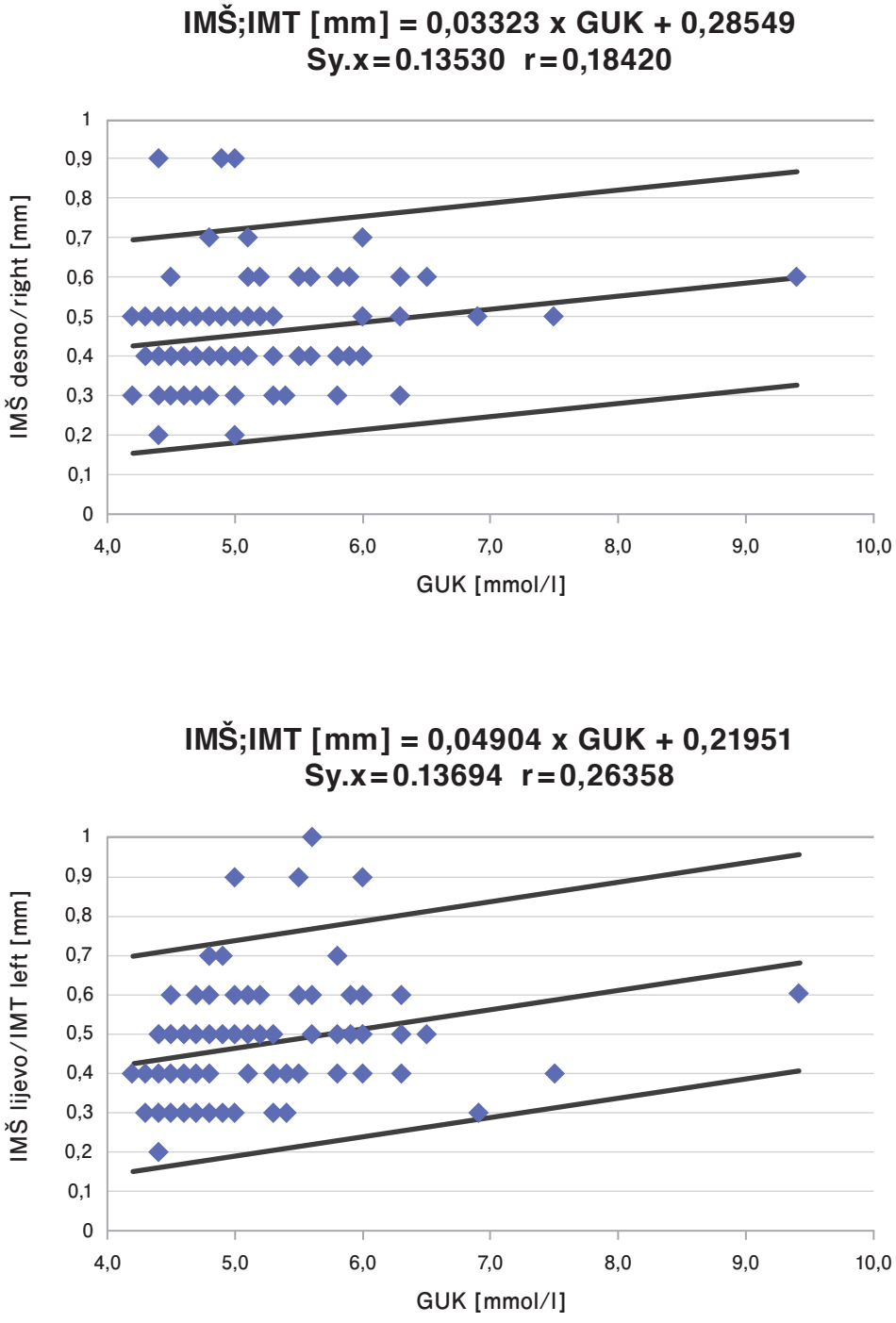

Fig. 7. **Correlation between CCA-IMT and fasting glucose level.**

Right side: correlation is positive but not statistically significant; r = 0.184, p = 0.071, CI = 95%. Left side: correlation is positive and statistically significant; r = 0.264, p = 0.009, CI = 95%. Subjects; n = 97 women, non-diabetics, age range 17-92, average age 53.42 ± 16.59 years. GUK = glucose; IMŠ lijevo = IMT left; IMŠ desno = IMT right; CI, confidence interval; CCA, common carotid artery; IMT, intima-media complex thickness.

Results

The results, as presented by graphs (***Fig. 7***), indicate well the visible linear positive correlation between fasting glucose level and CCA-IMT of the lower arterial wall. On the left side there was a statistically significant correlation: Pearson coefficient r is 0.264, $p = 0.009$, $p < 0.05$, Sy.x = 0.13694; on the right side the correlation was positive, but not statistically significant: $r = 0.184$, $p = 0.071$, $p > 0.05$, Sy.x = 0.13530. Confidence interval (CI) on both sides is 95%. Linear regression equation on the right side: CCA-IMTr = 0.03323*Glu. + 0.28549; Linear regression equation on the left side: CCA-IMTl = 0.04904*Glu. + 0.21951; It is clearly visible that the elevated glucose level was in the range of euglycemia (4-5 mmol/L, 80-110 mg/dL), which was true of the results of many of the examinees [1-4]. It was correlated with the elevated values of CCA-IMT.

Discussion

A number of recent investigations on the etiology of carotid atherosclerosis indicate a strong positive correlation between the CCA-IMT values and the course of local and generalised atherogenesis. The deviation to greater values in relation to the reference values according to age, indicate the possible already existing disease or a high risk of appearance of this disease in the future. These investigations based on the epidemiologic, clinical and laboratory approaches, show the relationship between CCA-IMT and the complex of conventional risk factors for atherosclerosis. Elevated values of blood pressure, low-density-lipoprotein (LDL) and very-low-density-lipoprotein (VLDL), glucose, BMI, triglicerides, insulin resistance, tobacco smoke and homocisteine, along with low high-density-lipoprotein (HDL), form the complex of risk factors which are under intensive analysis. At the same time, the atherogenic role of ROS and AGEs is also of topical interest [3,6,7].

Our results, which show a positive linear statistically significant (left CCA) correlation between CCA-IMT and FPG, are in agreement with the results of numerous studies on the same problem. Esposito *et al.* [2] state that postprandial hyperglycemia, but not fasting hyperglycemia, induces an evident increase in CCA-IMT. They have found that in the therapy of patients with Type 2 diabetes (during twelve months), repaglinide (Reglinid, Reodon) is much more effective in reducing CCA-IMT than glyburide (Daonil, Euglucon, Glibenclamide). A CCA-IMT decrease of > 0.020 mm was observed in 52% of diabetics receiving repaglinide and in 18% of those receiving glyburide ($P < 0.01$). This decrease in CCA-IMT was related only to the reduction of postprandial hyperglycemia but it was not related to fasting hyperglycemia.

Wagenknecht *et al.* [14] in their study on insulin resistance and atherosclerosis (IRAS – The Insulin Resistance Atherosclerosis Study), in a sample of verified Type 2 diabetes patients revealed positive correlation between fasting glucose level and CCA-IMT ($p < 0.05$). This was not found with the ICA-IMT (internal carotid artery intima-media thickness). In relation to the newly detected diabetics, the already verified diabetics have a 70 micron thicker CCA-IMT. The duration of Type 2 diabetes gave a positive but not statistically significant correlation with CCA-IMT. They concluded that chronic hyperglycemia induces an elevated risk for atherosclerosis. Therefore, they emphasise the great importance of primary and secondary prevention of diabetes. Hegazi *et al.* [15] in an analysis of 52 adipose men and women with Type 2 diabetes also found a correlation between CCA-IMT and hyperglycemia. Lee *et al.* [16] also obtained a positive correlation. Ciccone *et al.* [17] analysed this relation between FPG and CCA-IMT in a sample of 104 obese women of which 30 had Hashimoto thyroiditis (chronic hypothyroidism) and 74 had normal thyroid function. Univariate (simple) linear regression analysis, in these 74 women, revealed r of 0.25, $p = 0.03$, very similar to our results (rl of 0.264, $p = 0.009$). Both correlations were positive and statistically significant. None of these women had fasting glycemia levels of > 126 mg/dL (7.0 mmol/L). Our figures and their diagrams showing the regression lines are almost identical.

Selvin *et al.* [18] found strongly positive and statistically significant correlation between fasting glucose and HbA1c. Between these two parameters, and the risk for cardiovascular disease, there was also a positive relation. In addition, they found a positive correlation with CCA-IMT. Chronic hyperglycemia in diabetes is an independent risk factor for atherosclerosis development. They emphasise the importance of regular glycemia monitoring and HbA1c control. Kim *et al.* [19] present the correlation between HbA1c variability and CCA-IMT progression. They suggest that the progression of CCA-IMT could be associated with this variability but only after twelve months. They also propose that a prolonged observation is necessary for a precise definition. According to their conclusion, frequent hypoglycemic attacks induce the predisposition for damaging cardiovascular events. Homogenous fasting glucose values have much less harmful effects than variable values. The authors give the preference to postprandial glucose levels in relation to fasting glucose, as the reason for damaging effects. Pannacciulli *et al.* [20] in the univariate (simple) correlation in a similar investigation obtained an r value of $r = 0.24$; $p < 0.001$. They define CCA-IMT thickening as a surrogate marker of coronary atherosclerosis. Kozàkovà *et al.* [21] found r of 0.25; $p < 0.05$. Both r values are practically identical to our left r. In their recent study, Kozàkovà *et al.* [22] present results connected with the analysis of a European cohort of 525 men and 655 women (mean age 44 ± 8 years) free of conditions known to affect the carotid wall. They found that in women CCA-IMT is independently associated with FPG and emphasise that the relative cardiovascular risk associated with plasma glucose level is higher for non-diabetic women than men. They also present the fact that women with higher fasting glucose levels in the range of normoglycemia (5.2-6.8 mmmol/L), have a significantly higher leptin to adiponectin ratio as compared with women with lower glycemia values. Leptin (see ***Fig. 1***) is a strong proatherogenic factor (stimulation of an inflammatory reaction and vascular smooth muscle cell proliferation). However, adiponectin has opposite effects (attenuation of an inflammation, inhibition of proliferation of smooth muscle cells induced by growth factors). It seems, with great probability, that hyperglycemia and the elevated levels of euglycemia, directly impact leptin signaling (hexosamine biosynthetic pathway). It is important to mention that in normal physiology, leptin secretion is

higher in women than in men. Also, there is a higher percentage of obese tissue in women.

FPG and postprandial hyperglycemia in nondiabetics are probably usually too low to be able to significantly give rise to the glycation, AGEs generation, and endothelial injury with thickening of intima and media in the carotid artery wall. The above mentioned processes are present but clearly insufficient. In the case of diabetes, FPG and in particular postprandial hyperglycemia, are usually clearly elevated, and the discussed processes are much stronger and visible. Statistical correlation is also greater and in many studies significant [23-26].

The primary aim of our study was to present the fundamentals of glucose effects on the complex course of inter-and intramolecular events, which slowly, persistently and unstoppably lead to the irreparable damage of the arterial wall structures. Such damage is characterised by a rise of rigidity, thickness, inelasticity, drop of compliance, and lastly with the occurrence of atherosclerotic plaque. The essential process in these events is related to the marked accumulation of AGEs on long protein chains of the arterial wall, primarily on collagen. The aldehyde groups of reducing sugars (glucose, glucose-6-phosphate, fructose, ribose) nonenzymatically link with amino groups of protein side chains, and induce the generation composed compounds, *i.e.* Schiff base and Amadori product (early glycation products). It is clear that glucose is not an inert biological species, but rather it is capable of reacting with proteins, lipids and nucleic acids (***Fig. 1-4***) [3,7-11].

Glycation (non-enzymatic glycosylation) is a reaction of typical covalent bonding between the protein amino group (-NH_2) and the carbonil group (C=O) of sugar (glucose, fructose). The result is the unstable Schiff base and enaminol (1,2-enaminol intermediate-primary step of the Maillard reaction), and the relatively stable Amadori product. By oxidation and autoxidation, it yields many dangerous ROS products. Elevated chronic glucose levels through protein kinase C (PKC) in the diacylglycerol (DAG)-PKC pathway, NAD(P)H and mitochondria produce an electron excess and consequent ROS generation (possible oxidative stress). Elevated H_2O_2 production via the Fenton reaction generates very dangerous OH* (hydroxyl radical). Hyperglycemia also induces the production of peroxinitrite $ONOO^-$ (ROS). Chronic elevation of glucose levels, through the production of glucosone and glyoxal (reactive intermediates), as well as through N^{ε}-(carboxyethyl)-lysine (CML) formation and receptor for AGEs (RAGE), accelerates atherogenesis (***Fig. 1***) [7].

Amadori product dehydration through Amadori dione cross link, stimulates collagen cross linking with a loss of its (collagen) function. The final result is an increase in arterial wall stiffness, rigidity, and thickness, and a loss of elasticity and decreased compliance (***Fig. 2***) [7].

Glycation between glucose and a phospholipid with an amine component of the LDL particle, drastically changes the LDL function and accelerates atherogenecity. Glycated LDL plays a role in completely different relations with many important cell receptors. Of particular importance is the newly revealed, very grim situation of LDL receptors (LDLR) in the liver. LDL particle recognition and LDLR binding function is dramatically deranged. LDLR in the liver cannot recognise, bind and neutralise glycated and oxidised very dangerous LDL particles. They continue to flow in the blood. The complete situation is presented in ***Fig. 3*** [9].

By phosphorylation and activation of the IκB kinase (IKK) complex, (IKK-a, IKK-b and IKK-g heterodimers), the phosphorylation and degradation of IκB protein (strong blocker of NF-κB kinase) is strongly activated. Liberated NF-κB quickly translocates into the nucleus and induces the transcription of numerous atherogenic genes [platelet-derived growth factor beta (PDGF-B), vascular cell adhesion molecule-1 (VCAM-1), E-selectin, monocyte chemoattractant protein-1 (MCP-1), cyclooxygenase-2 (COX-2), matrix metalloproteinase-2 (MMP-2), interleukin-8 (IL-8)] (***Fig. 4***) [27].

The early phase of protein glycation (Maillard reaction) generates the Amadori product. This process in our body is very slow and is dependent on the chronic high glucose level and the half-life of protein molecules. As the half-life of collagen is very long (cartilage collagen 117 years, skin collagen 15 years), it is clear why this process is so slow. The late phase of the transformation of these compounds can go into three varied directions: glucose autoxidation through glyoxal, the cleaving of the Schiff base through the Namiki pathway, and oxidative cleavage of the Amadori product by reactive oxygen species (ROS). All research has indicated that the third direction of the late transformation in the above-mentioned AGEs *in vivo* is dominant. A number of studies show that hyperglycemia through NAD(P)H oxidase activation and mitochondrial electron transport chain (ETC), leads to marked generation of superoxide anion radical (O_2^{*-}). The reaction of these radicals with hydrogen (H), produces hydrogen peroxide (H_2O_2), and the Fenton reaction yields the hydroxyl radical (OH*). In contrast, O_2^{*-} binds with nitrogen oxide (NO) and yields peroxynitrite $ONOO^-$. Both of these compounds are ROS with a strong oxidative capacity. They oxidatively cleave the Amadori product (early glycation product) into the complex AGE compound CML [3,7].

Without entering into speculation about the structure and effects of AGE-complex compounds, the aim of this study was to primarily present the aforementioned CML and its effects. This presents the complex of a cleaved part of the Amadori product and side epsilon (ε) amine residue of lysine, *i.e.* hydroxylysine, one component of collagen. It is important to note that this compound is not a crosslinker of protein molecules. The dominant effect of this compound is the binding to RAGE and its strong activation with numerous biological responses. Of particular importance is the NF-κB activation with its signal cascade which promotes a strong atherogenic response. Activated NF-κB quickly translocates from the cytoplasm to the nucleus with transcriptional activation of many important genes, especially those for cytokine transcription. NF-κB is found in high concentrations in atherosclerotic lesions in the intima and media. The strong expression of VCAM-1, intercellular adhesion molecule-1 [ICAM-1], MCP-1, COX-2,and MMP-2 leads to proliferation and migration of vascular smooth muscle cells (VSMCs) in the media, monocyte attraction from the blood into the arterial wall, and secretion of many cytokines. The result is an increase in CCA-IMT. AGE-linking on the long protein molecules (collagen) results in arterial wall rigidity, a drop

in elasticity and decreased compliance. The local pulse pressure rises, intracellular actin structure distends, mitochondrial activity is elevated, and O_2^{*-} rises [3,7,13,28].

In addition to CML, which is not a crosslinker, numerous other AGE-compounds are strong crosslinkers of long protein molecules. In this way these AGE compounds disturb normal function and lead to pathological conditions [7].

Amadori product generates O_2^{*-} and H_2O_2 which via the Fenton reaction, result in OH* production. Amadori product is subject to numerous changes. Its dehydration through the intermediates amadori dione and amadori enedione generates amadori dione cross-link, a strong cross-linking factor. Glucose, Schiff base, and Amadori product, can be transformed into reactive intermediates (glycolaldehyde, glyoxal, glucosone, methylglyoxal diimine, 3-deoxyglucosone), *i.e.* reactive aldehydes, which by formation of AGE compounds glyoxal-lysine dimer [GOLD], methylglyoxal-lysine dimer [MOLD], CML, pyrraline, crossline, pentosidine) lead to the protein cross-linking (collagen elastin, crystallin) and alteration of their function. Numerous laboratory tests on rats show that AGE-inhibitors have a beneficial effect on the course of retinopathy, neuropathy and nephropathy in experimentally-induced diabetes. Those compounds (aminoguanidine, ALT-711: Alteon-Alagebrium chloride-strong cross-linker breaker, DPTC: dimethyl-3-phenylacylthiazolium chloride-strong protein-protein cross-linker breaker) as a set of antioxidants and free radical scavengers (for example, *Ginkgo biloba*) are recently under intensive investigations with the aim to determine the optimal means to combat AGE-effects [7,9,28-36]. Deteriorative effects of PKC are also under intensive investigations [7,37-40].

AGEs are primarily generated in our body, but they are also important components in our foods. Foods rich in proteins and lipids are abundant in AGEs. On the other hand, foods rich in carbohydrates, such as fruits, vegetables, milk, and cereals, have only a small quantity of AGEs. Prolonged food thermal processing at high temperatures (grilling, roasting) produces a large quantity of AGEs. Dry heat is particularly dangerous, as is sugar carmelisation. Unfortunately, the food processed via the above-mentioned methods resulting in an abundance of AGEs, is very tasty. Present-day cooking, which is inconcivable without termal processing, instigates the increasing occurrence of diabetes and cardiovascular diseases. [3,7,41]. In his paper on Alzheimer's disease, Barić [42] has clearly presented a variety of practical procedures during food processing which are beneficial for AGE protection. Some of them are mentioned above.

The results of this study clearly demonstrate that even in the range of normoglycemia (80-110 mg/dL, 4-6 mmol/L), small differences in fasting glucose levels are in positive linear correlation with the values of CCA-IMT. The analysis of Nielson and Fleming [4] with their sample of nondiabetics strongly correlates with the present results. In their sample of nondiabetics with elevated values of fasting normoglycemia, they found a statistically significant high incidence of transient ischemic attack (TIA) and stroke.

Glucose (D-glucose), the object of this study, is a monosaccharide (monomolecular sugar), hexose (six C atoms), aldose (simple sugar with one aldehyde group), and reductant (electron donor). Glycation of proteins is possible through glucose with an open chain (open-chain or extended "stickman" form-Fischer projection) and not through glucose with a cyclic structure (Haworth projection form). In normal physiology there is an equilibrium between these two forms (99 %, < 1 %). Their imbalance can accelerate glycation [43].

In the end, it is necessary to emphasize that AGEs are permanently generated in our body regardless of the optimal conditions of normoglycemia. There is no such low border of glycemia at which the AGE generation is completely terminated. Glycation is a normal component of our body physiology; it is inevitable. It takes place both intra-and extracellularly. In diabetes it is accelerated. In parallel with the above-mentioned AGE generation, AGEs unstoppably enter our body via different foods. This study is, however, focused on the elements of conditionally normal, physiological aging (aging without diseases). Regular prevention and adequate therapy of diabetes and its complications, as well as the battle against AGEs and ROS, is a great task for modern medicine. This is also relevant to all other conditions that lead to hyperglycemia and its harmful effects [3,7].

Conclusion

Carotid atherosclerosis is a very serious and dangerous disease. There is a great necessity for improving preventive and therapeutic methods to lower its occurrence and harmful consequences. The fasting glucose level in hyperglycemia and also in normoglycemia, according to a number of investigations, has a strong positive correlation with CCA-IMT. CCA-IMT is a strong predictor of actual local and generalised atherosclerosis. Every aberration of its values from the standard values typical for examinee age can be ubious; this requires a detailed elaboration of the individual patient. The battle against elevated glucose levels, especially in diabetics, and against AGEs, as crucial deteriorative factors connected with hyperglycemia, should be a continuous effort in physicians' daily activities.

Conflict of interest statement

The authors have no conflict of interest related to this study to declare.

References

1) Aronson D, Rayfield EJ. How hyperglycemia promotes atherosclerosis: Molecular mechanisms. Cardiovasc Diabetol. 2002; 1: 1.
2) Esposito K, Ciugliano D, Nappo F, et al. Regression of carotid atheroscle-rosos by control of postprandial hyperglycemia in type 2 diabetes mellitus. Circulation. 2004; 110: 214-219.
3) Peppa M, Uribarri J, Vlassara H. Glucose, advanced glycation end products, and diabetes complications: What is new and what works. Clinical Diabetes. 2003; 21: 186-187.
4) Nielson C, Fleming RM. Blood glucose and cerebrovascular disease in nondiabetic patients. Angiology. 2007; 58: 625-629.
5) Barić N, Materljan E, Barić-Santro S. Korelacija između životne dobi i debljine intima-medija. kompleksa zajedničke karotidne arterije kao mark-era aterogeneze. U: Mazzi B. ur.Zbornik-Sedmi kongres HDOD-HLZ.. Rovinj 2007; str. 111-28.
6) Goldin A, Beckman JA, Schmidt AM, et al. Advanced glycation end prod-ucts: Sparking the development of diabetic vascular injury. Circulation. 2006; 114: 597-605.
7) Nagai R. Accumulation of advanced glycation end products in age-dependent disorders. Med Hypotheses Res. 2004; 1: 161-170.
8) Alikhani M, MacLellan CM, Raptis M, et al. Advanced glycation end pro-ducts induce apoptosis in fibroblasts through activation of ROS, MAP kinases, and the FOXO1 transcription factor. Am J Physiol Cell Physiol. 2007; 292: C850-856.
9) Nagai R, Matsumoto K, Ling X, et al. Glycolaldehyde, a reactive intermedi-ate for advanced glycation end products, plays an important role in the generation of an active ligand for macrophage scavenger receptor. Diabetes. 2000; 49: 1714-1723.
10) Reiser KM. Nonenzymatic glycation of collagen in aging and diabetes. Proc Soc Exp Biol Med. 1998; 218: 23-37.
11) Nagai R, Unno Y, Hayashi MC, et al. Peroxynitrite induces formation of Nε-(carboxymethyl)-lysine by the cleavage of Amadori product and generation of glucosone and glyoxal from glucose: Novel pathways for protein modification by peroxynitrite. Diabetes. 2002; 51: 2833-2839.
12) Hayashi CM, Nagai R, Miyazaki K, et al. Conversion of Amadori products of the Maillard reaction to N^{ε}-(carboxymethyl) lysine by short-term heating: Possible detection of artifacts by immunohistochemistry. Lab Invest. 2002; 82: 795-808.
13) Barić N, Materljan E, Juretić M, et al. Utjecaj procesa starenja na odnos širina intima-medija/unutrašnji promjer u zajedničkoj karotidnoj arteriji. U: Mazzi B. ur. Zbornik-Osmi kongres HDOD-HLZ. Rovinj 2008; str. 77-94.
14) Wagenknecht LE, D'Agostino R Jr, Savage PJ, et al. Duration of diabetes and carotid wall thickness: The insulin resistence atherosclerosis study (IRAS). Stroke. 1997; 28: 999-1005.
15) Hegazi RA, Sutton-Tyrreell K, Evans RW, et al. Relationship of adiposity to subclinical atherosclerosis in obese patients with type 2 diabetes. Obes Res. 2003; 11: 1597-1605.
16) Lee EJ, Kim HJ, Bae JM, et al. Relevance of common carotid intima-media thickness and carotid plaque as risk factors for ischemic stroke in patients with type 2 diabetes mellitus. AJNR. 2007; 28: 916-919.
17) Ciccone MM, De Pergola G, Porcelli MT, et al. Increased carotid IMT in overweight and obese women affected by Hashimoto's thyroiditis: An adi-posity and autoimmune linkage? BMC Cardiovasc Disord. 2010; 10: 22.
18) Selvin E, Coresh J, Golden SH, et al. Glycemic control, atherosclerosis, and risk factors for cardiovascular disease in individuals with diabetes: The atherosclerosis risk in communities study. Diabetes Care. 2005; 28: 1965-1973.
19) Kim CS, Park SY, Yu SH, et al. Is A1C variability an independent predictor for the progression of atherosclerosis in type 2 diabetic patients? Korean Diabetes J. 2010; 34: 174-181.
20) Pannacciulli N, De Pergola G, Ciccone M, et al. Effect of family history of type 2 diabetes on the intima-media thickness of the common carotid artery in normal-weight, overweight, and obese glucose-tolerant young adults. Diabetes Care. 2003; 26: 1230-1234.
21) Kozàkovà M, Palombo C, Morizzo C, et al. Effect of sedentary behaviour and vigorous physical activity on segment-specific carotid wall thickness and its progression in a healthy population. Eur Heart J. 2010; 31: 1511-1519.
22) Kozàkovà M, Natali A, Dekker J, et al. Insulin sensitivity and carotid intima-media thickness: Relationship between insulin sensitivity and cardiovascu-lar risk study. Arterioscler Thromb Vasc Biol. 2013; 33: 1409-1417.
23) Semba RD, Beck J, Sun K, et al. Relationship of a dominant advanced gly-cation end product, serum carboxymethyl-lysine, and abnormal glucose me-tabolism in adults: The Baltimore Longitudinal Study of Aging. J Nutr Health Aging. 2010; 14: 507-513.
24) Teichert T, Hellwig A, Peβler A, et al. Association between advanced glyca-tion end products and impaired fasting glucose: Results from the SALIA Study. PloS One. 2015; 10(5): e0128293.
25) Node K, Inoue T. Postprandial hyperglycemia as an etiological factor in vascular failure. Cardiovasc Diabetol. 2009; 8: 23.
26) Kowall B, Ebert N, Then C, et al. Associations between blood glucose and carotid intima-media thickness disappear after adjustment for shared risk factors: The KORA F4 study. PloS One. 2012; 7(12): e52590.
27) Inoguchi T, Li P, Umeda F, et al. High glucose level and free fatty acid stimulate reactive oxygen species production through protein kinase C-dependent activation of NAD(P)H oxidase in cultured vascular cells. Diabe-tes. 2000; 49: 1939-1945.
28) Baumann M, Richart T, Sullinger D, et al. Association between carotid diameter and advanced glycation end product N^{ε}-carboxymethyllysine (CML). Cardiovasc Diabetol. 2009; 8: 45.
29) Bakris GL, Bank AJ, Kass DA, et al. Advanced glycation end-product cross-link breakers. A novel approach to cardiovascular pathologies related to the aging process. Am J Hypertens. 2004; 17: 23S-30S.

30) Asif M, Egan J, Vasan S, et al. An advanced glycation endproduct cross-link breaker can reverse age-related increases in myocardial stiffness. Proc Natl Acad Sci USA. 2000; 97: 2809-2813.
31) Liu J, Masurekar MR, Vatner DE, et al. Glycation end-product cross-link breaker reduces collagen and improves cardiac function in aging diabetic heart. Am J Physiol Heart Circ Physiol. 2003; 285: H2587-H2591.
32) Vaitkevicius PV, Lane M, Spurgeon H, et al. A cross-link breaker has sus-tained effects on arterial and ventricular properties in older rhesus monkeys. Proc Natl Acad Sci USA. 2001; 98: 1171-1175.
33) Ulrich P, Cerami A. Protein glycation, diabetes, and aging. Recent Prog Horm Res. 2001; 56: 1-22.
34) Demarin V, Podobnik SS, Storga-Tomić D, et al. Treatment of Alzheimer's disease with stabilized oral nicotinamide adenine dinucleotide: A random-ized, double-blind study. Drugs Exp Clin Res. 2004; 30: 27-33.
35) Ziemen SJ, Kass DA. Advanced glycation end product cross-linking: Patho-physiologic role and therapeutic target in cardiovascular disease. Congest Heart Fail. 2004; 10: 144-151.
36) Semba RD, Najjar SS, Sun K, et al. Serum carboxymethyl-lysine, an ad-vanced glycation end product, is associated with increased aortic pulse wave velocity in adults. Am J Hypertens. 2009; 22: 74-79.
37) He Z, King GL. Protein kinase Cβ isoform inhibitors: A new treatment for diabetic cardiovascular diseases. Circulation. 2004; 110: 7-9.
38) Inoguchi T, Sonta T, Tsubouchi H, et al. Protein kinase C-dependent in-crease in reactive oxygen species (ROS) production in vascular tissues of diabetes: Role of vascular NAD(P)H oxidase. J Am Soc Nephrol. 2003; 14: S227-S232.
39) Inoguchi T, Nawata H. NAD(P)H oxidase activation: A potential target mechanism for diabetic vascular complications, progressive beta-cell dys-function and metabolic syndrome. Curr Drug Targets. 2005; 6: 495-501.
40) Geraldes P, King GL. Activation of protein kinase C isoforms and its impact on complications. Circ Res. 2010; 106: 1319-1331.
41) Uribarri J, Woodruff S, Goodman S, et al. Advanced Glycation end products in foods and a practical guide to their reduction in diet. J Am Diet Assoc. 2010; 110: 911-916.
42) Barić N. Role of advanced glycation and products in Alzheimer's disease. Glycative Stress Research. 2014; 1: 68-83.
43) Wang Y, Yu H, Shi X, et al. Structural mechanism of ring-opening reaction of glucose by human serum albumin. J Biol Chem. 2013; 288: 15980-15987.