Available online at www.sciencedirect.com
ScienceDirect
Metabolism Clinical and Experimental 57 (2008) 1211 – 1220
Metabolism
Clinical and Experimental
www.metabolismjournal.com

Methylglyoxal production in vascular smooth muscle cells from different metabolic precursors

Arti Dhar[a], Kaushik Desai[a], Michael Kazachmov[b], Peter Yu[a,b], Lingyun Wu[a,*]

[a]*Department of Pharmacology, College of Medicine, University of Saskatchewan, Saskatoon, SK, Canada S7N 5E5*
[b]*Department of Neuropsychiatry Research Unit, College of Medicine, University of Saskatchewan, Saskatoon, SK, Canada S7N 5E5*

Received 16 October 2007; accepted 24 April 2008

Abstract

Methylglyoxal (MG), a metabolic by-product, reacts with certain proteins to yield irreversible advanced glycation end products (AGEs) and increases oxidative stress that causes the pathophysiological changes in diabetes, hypertension, and aging. Although MG production from glucose has been well documented, the contribution of other intermediates of different metabolic pathways to MG formation is far less known. Our aim was to determine and compare the formation of MG, MG-induced AGE, N^{ε}-carboxyethyl-lysine (CEL), inducible nitric oxide synthase (iNOS), nitric oxide, and peroxynitrite from different metabolic precursors in cultured rat aortic vascular smooth muscle cells (VSMCs). High-performance liquid chromatography was used to determine MG levels, whereas nitrite + nitrate, indicators of nitric oxide production, and peroxynitrite levels were measured with specific assay kits. The CEL and iNOS were detected using immunocytochemistry. There was a concentration-dependent increase in MG levels in VSMCs after 3-hour incubation with 5, 15, and 25 mmol/L of D-glucose, fructose, or aminoacetone. Aminoacetone produced a 7-fold increase in MG levels above the basal value followed by fructose (3.9-fold), D-glucose (3.5-fold), acetol (2.8-fold), and sucrose (2.3-fold) after a 3-hour incubation with 25 mmol/L of each precursor. L-Glucose, 3-*O*-methylglucose, and mannitol had no effect on MG production. All precursors, except L-glucose, 3-*O*-methylglucose and mannitol, increased CEL. Aminoacetone, D-glucose, and fructose significantly increased iNOS, nitrite/nitrate, and peroxynitrite levels. In conclusion, aminoacetone is the most potent precursor of MG production in VSMCs, followed by fructose and D-glucose. This could have important implications in relation to high dietary fructose and protein intake.

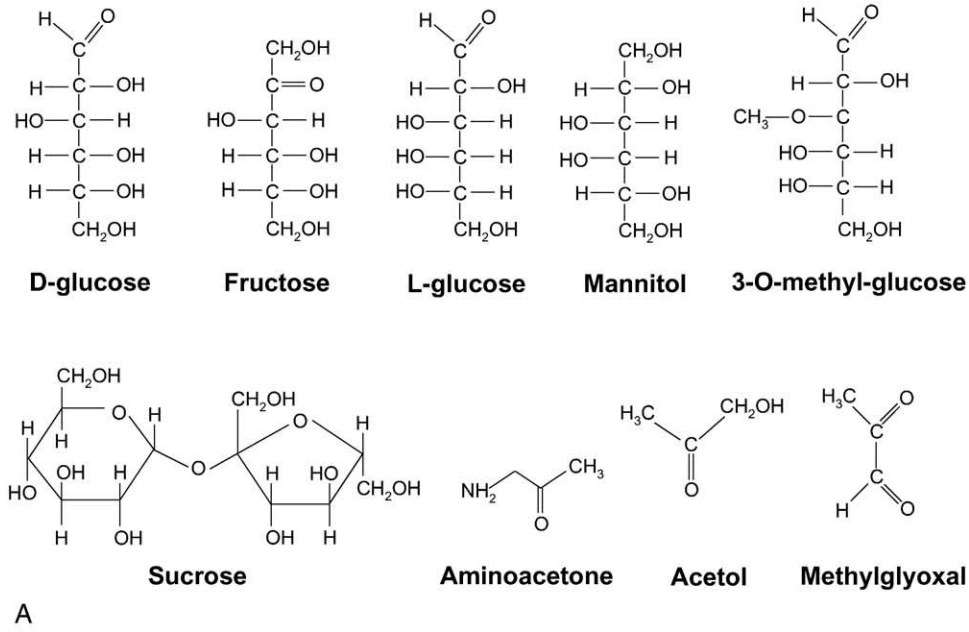
1. Introduction

A number of highly reactive metabolic intermediates of glucose, fatty acids, and amino acids react with proteins, lipids, DNA, and other molecules and alter their normal structure and/or function [1]. Methylglyoxal (MG) is one such highly reactive dicarbonyl compound formed mainly in the glycolytic pathway nonenzymatically [2,3] (Fig. 1A, B). Other sources of MG include intermediates of protein metabolism, such as aminoacetone [4], and fatty acid metabolism, such as acetone and acetol [5] (Fig. 1A, B). The enzymes catalyzing conversion of aminoacetone and acetone/acetol to MG are semicarbazide-sensitive amine oxidase (SSAO) [6] and acetone monooxygenase/acetol monooxygenase [5], respectively. The SSAO is found in vascular smooth muscle cells (VSMCs) and the plasma [6].

Methylglyoxal reacts oxidatively with arginine or lysine residues of proteins and forms irreversible advanced glycation end products (AGEs) such as argpyrimidine [7], N^{ε}-carboxymethyl-lysine (CML) [8], and N^{ε}-carboxyethyl-lysine (CEL) [9]. Along with 3-deoxyglucosone and glyoxal, MG is a major source of intracellular and plasma AGEs [10]. Elevated MG levels and AGEs such as CEL and CML are markers of carbonyl overload, oxidative stress, and the resultant damage in aging and diabetes [10]. An increase in glucose leads to increased formation of MG as occurs in diabetes [2,10]. The serum concentration of MG increases in patients with type 1 or type 2 diabetes mellitus [11,12]. We have recently shown that MG attaches to the internal arginine in the β-chain of insulin [13] and significantly reduces its capacity to stimulate [^{3}H]-2-deoxyglucose uptake by 3T3-L1 adipocytes and L8 skeletal muscle cells, compared with native insulin. These structural and functional abnormalities of insulin may contribute to the pathogenesis of insulin resistance [13]. Our studies have also shown that as

* Corresponding author. Tel.: +1 306 966 6310; fax: +1 306 966 6220.
E-mail address: lily.wu@usask.ca (L. Wu).

0026-0495/$ – see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2008.04.014



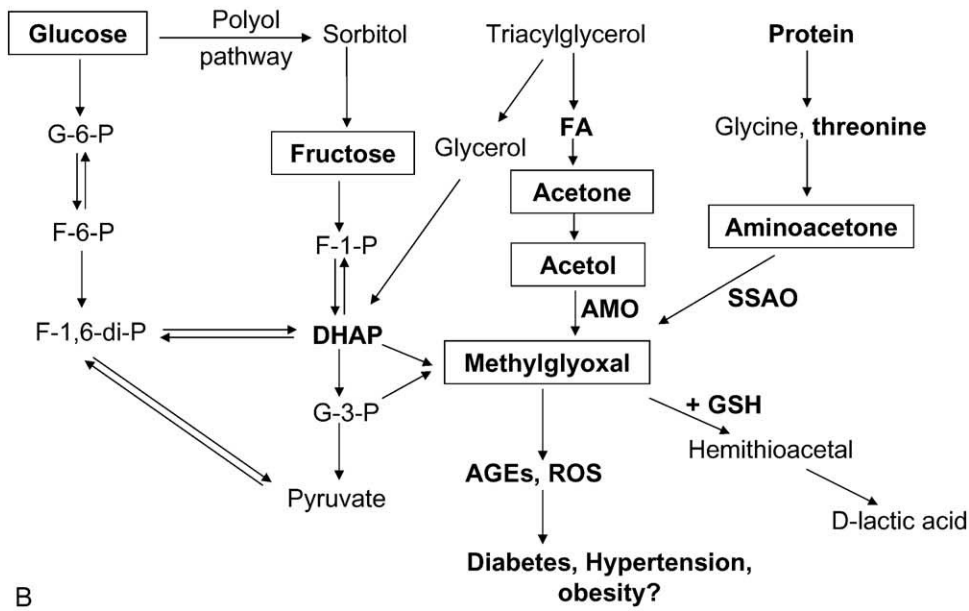


Fig. 1. A, Structures of MG and various metabolic precursors used for the study. B, Pathways of MG formation from different substrates. AMO indicates acetone monooxygenase and acetol monooxygenase; DHAP, dihydroxyacetone phosphate; FA, fatty acid; F-1-P, fructose-1-phosphate; F-1,6-di-P, fructose-1,6-bisphosphate; F-6-P, fructose-6-phosphate; G-6-P, glucose-6-phosphate; G-3-P, glyceraldehyde-3-phosphate; ROS, reactive oxygen species.

spontaneously hypertensive rats developed hypertension, there was an increase in MG levels in the plasma and aorta in an age-dependent fashion. No difference was observed in blood glucose levels between spontaneously hypertensive rats and normotensive Wistar-Kyoto rats [14,15].

We have shown earlier that MG activated nuclear factor κB p65 and increased oxidative stress in rat VSMCs [16]. Methylglyoxal induced inducible nitric oxide synthase (iNOS) and increased formation of peroxynitrite, a highly reactive oxidant that was inhibited by the NOS inhibitor *N*ω-nitro-L-arginine methyl ester [17,18]. Increased peroxynitrite has been found in type 1 diabetes mellitus [19]. Superoxide, hydrogen peroxide, and peroxynitrite activate nuclear factor κB, which triggers inflammation and proliferative responses in VSMCs [20].

Glucose, the principal carbohydrate nutrient in the body, is also the most important contributor to the formation of glycated proteins [1,2,10]. Fructose, another monosaccharide consumed in our diet, is also a precursor of MG [18]. Sucrose from the diet is converted into glucose and fructose. Metabolic intermediates such as glucose-6-phosphate form AGEs at a faster rate than glucose [21].

Given the importance of endogenous MG level and the potential for MG production from various metabolic intermediates of carbohydrates, proteins, and fatty acids (Fig. 1B), it is imperative to characterize the transformation capability of different metabolic precursors to MG in the cells. The production of MG from glucose has been well documented and quantified [2,3,10]. Unfortunately, production of MG from other metabolic precursors, such as aminoacetone, acetol, or sucrose, has not been systematically and directly examined or quantitatively compared with that from glucose. Our main aim was to determine and compare the formation of MG from different precursors (Fig. 1A, B) in VSMCs to provide fundamental information for cellular MG production. The MG-induced CEL, iNOS, NO, and peroxynitrite in the presence of different metabolic precursors were further investigated in VSMCs.

2. Methods

2.1. VSMC culture

Rat thoracic aortic smooth muscle cell line (A-10 cells) was obtained from American Type Culture Collection and cultured in Dulbecco modified Eagle medium (DMEM) containing 10% fetal bovine serum (FBS) at 37°C in a humidified atmosphere of 95% air and 5% CO_2, as described in our previous studies [17,18]. The A-10 cells were seeded either in 100-mm dishes for MG measurement or in 96-well plates for other assays, with an equal amount of cells (10^6/mL) in each well, and cultured to confluence. For immunocytochemistry staining, cells were seeded on cover glass slides (2×10^6/mL). Cells were starved in FBS-free DMEM for 24 hours before exposure to different metabolic precursors.

2.2. MG measurement

Methylglyoxal was measured by a specific and sensitive high-performance liquid chromatography method [14,18]. Methylglyoxal was derivatized with *o*-phenylenediamine to form the quinoxaline product 2-methylquinoxaline, which is very specific for MG [14,18]. After the specified incubation time of the cultured cells with precursors such as glucose and fructose, the culture medium was aspirated completely; and the cells were washed twice with phosphate-buffered saline (PBS). The cells were scrapped, and cell pellets were resuspended in ice-cold PBS and lysed over ice by sonication (5 seconds, 3 times). The sample was then incubated on ice for 10 minutes with 1/4 vol of 1 N perchloric acid and centrifuged (12 000 rpm, 15 minutes) to remove the perchloric acid–precipitated material. The supernatant was supplemented with 10 mmol/L *o*-phenylenediamine and incubated for 3 hours at room temperature. The 2-methylquinoxaline and underived 5-methylquinoxaline, which was added to the samples as the internal standard, were quantified on a Hitachi D-7000 high-performance liquid chromatography system (Hitachi, Mississauga, Ontario, Canada) via Nova-Pak C18 column (3.9 × 150 mm, 4 μm particle diameter; Waters, Milford, MA).

2.3. Measurement of peroxynitrite

The formation of peroxynitrite was determined by a sensitive dicholorofluorescin assay as described previously [18]. Briefly, cells were loaded with a membrane-permeable, nonfluorescent probe 2′,7′-dichlorofluorescin diacetate (CM-H2DCFDA, 5 μmol/L) for 2 hours at 37°C in FBS-free DMEM in the dark. After washing with PBS 3 times, cells were treated with or without different substrates for 3 hours and finally subjected to detection. Once inside the cells, CM-H2DCFDA becomes membrane-impermeable dicholorofluorescin 2 in the presence of cytosolic esterases and is further oxidized mostly by peroxynitrite to form the fluorescent oxidized dichlorofluorescein (DCF). The probe has high reactivity with peroxynitrite and its products CO_3^- and NO_2 but is not entirely specific for it. It also has low reactivity for hydrogen peroxide and even lower for superoxide [22]. The fluorescence intensity was measured with excitation at 485 nm and emission at 527 nm using a Fluoroskan Ascent plate reader (ThermoLab System, Helsinki, Finland) and Ascent software and expressed in arbitrary units.

2.4. Measurement of nitrite and nitrate

Cells were incubated with different substrates for 3 hours and then washed with PBS. The supernatant was used for the measurement of nitrite and nitrate with a fluorimetric assay kit (Calbiochem-Novobiochem, San Diego, CA) based on the Greiss reaction. The assay is based on the enzymatic conversion of nitrate to nitrite by nitrate reductase followed by the addition of 2,3-diaminonaphthalene, which converts nitrite to a fluorescent compound. Fluorescence intensity measurements of this compound accurately determine the nitrite (NO_2) concentration (excitation maximum, 365 nm; emission maximum, 450 nm).

2.5. Immunocytochemistry

The A-10 cells were seeded on glass coverslips, incubated with different substrates for 3 hours, and subjected to iNOS and CEL staining. As described previously [18], the treated cells were fixed in 4% paraformaldehyde for 30 minutes at room temperature and washed twice with 0.01 N PBS. After permeation with 0.1% Triton X-100 for 5 minutes and 2 washes with PBS, the cells were incubated with normal goat serum (diluted 1:30 in 0.1 N PBS) for 1 hour to block nonspecific binding sites. After shaking off the goat serum, the slides were incubated with iNOS antibody (1:500; BD Transduction Laboratories, Lexington, KY) and CEL antibody (1:100; a generous gift from Novo Nordisk, Bagsvaerd, Denmark) overnight at room temperature. Cells were washed twice in PBS (0.01 N) for 5 minutes and incubated with secondary fluorescein isothiocynate–conjugated anti-CEL and Texas red–conjugated anti-iNOS antibodies (Molecular Probes; Invitrogen, Carlsbad, CA) for 2 hours. After washing thrice with PBS, the slides were mounted in glycerol-PBS (3:7), coverslipped, and observed under a fluorescence

microscope. Staining intensity was quantified using the Metamorph image analysis software (version 7; Molecular Devices, Sunnyvale, CA). Slides from 4 different experiments were analyzed with 5 fields per slide observed and averaged.

2.6. Materials

The substrates used in this study (Fig. 1A), D- and L-glucose, mannitol, fructose, sucrose, 3-*O*-methylglucose (3-OMG), and acetol, were purchased from BDH (Toronto, ON, Canada). Aminoacetone was synthesized and characterized in our laboratory as described previously [23]. All cell culture reagents were purchased from GIBCO (Grand Island, NY).

2.7. Data analysis

Data are expressed as mean ± SEM and analyzed using 1-way analysis of variance and post hoc Dunnett test where applicable. $P < .05$ was considered statistically significant when group difference was compared.

3. Results

3.1. Generation of MG from different metabolic precursors

Cultured VSMCs were incubated with D-glucose, fructose, and aminoacetone at different concentrations for 3 hours. The 3-hour incubation time was based on our previous observation of a time-dependent production of MG after incubation of VSMCs with fructose that reached a peak at 3 hours [18]. All 3 precursors induced a concentration-dependent increase in MG levels (Fig. 2A). The greatest increase was observed with aminoacetone, whereas fructose and D-glucose exhibited similar potency in generating MG (Fig. 2A, B). Even at 5 mmol/L, the amount of MG produced by aminoacetone is 3.6-fold more than that produced by an equimolar concentration of D-glucose and 3.9-fold above the baseline levels of MG (Fig. 2A).

In subsequent comparative experiments, in addition to D-glucose, fructose, and aminoacetone, acetol and sucrose also produced significant amount of MG in cultured VSMCs when 25 mmol/L of each substrate was incubated for 3 hours (Fig. 2B). However, L-glucose, 3-OMG, and mannitol at the same concentration of 25 mmol/L did not induce significant production of MG after 3-hour incubation of VSMCs (Fig 2B).

3.2. Effect of different MG precursors on CEL formation

The levels of the MG-induced AGE CEL were increased in VSMCs after incubation with aminoacetone, fructose, D-glucose, acetol, and sucrose, each at 25 mmol/L for 24 hours (Fig. 3A). The CEL staining was located mainly in the cytosol of the cell with all precursors except aminoacetone, which showed CEL over the nuclear area also. The greatest increase was induced by aminoacetone, followed by fructose and D-glucose (Fig. 3B). Acetol and sucrose caused less increase in CEL formation at a 25-mmol/L concentration incubated for

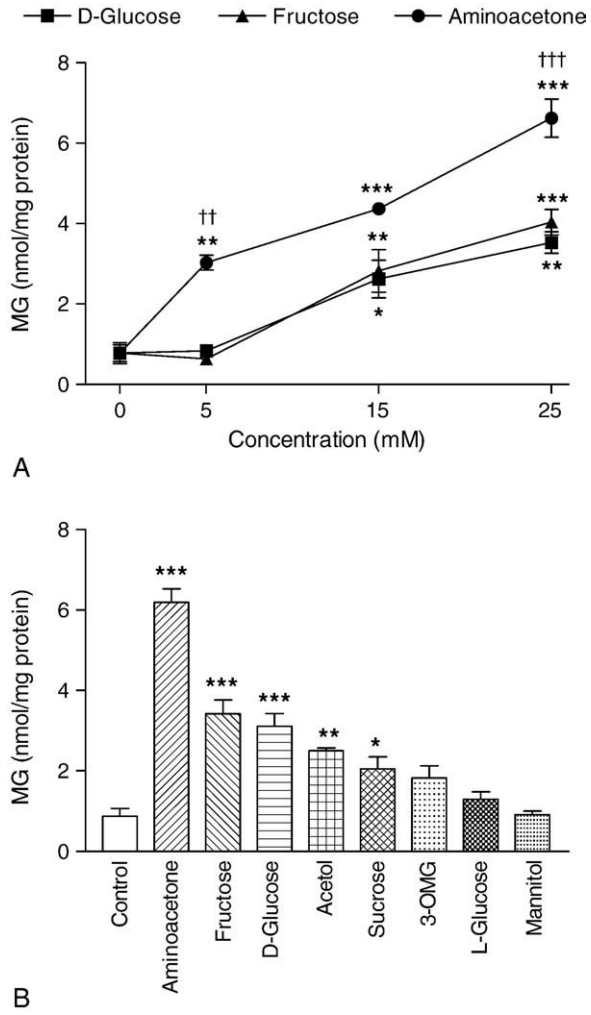


Fig. 2. A, Concentration-dependent production of MG from different metabolic precursors in cultured rat aortic smooth muscle cells (A-10). The production of MG was determined after A-10 cells were incubated for 3 hours with 5, 15, or 25 mmol/L of precursors. n = 3 to 4 for each group. $^{*}P < .05$, $^{**}P < .01$, and $^{***}P < .001$ vs basal value. $^{\dagger\dagger}P < .01$ and $^{\dagger\dagger\dagger}P < .001$ vs fructose and D-glucose at same concentration. B, Different amounts of MG are produced from different substrates in cultured rat aortic smooth muscle cells (A-10). The production of MG was determined after A-10 cells were incubated for 3 hours with different substrates (25 mmol/L each). n = 6 for each group. $^{*}P < .05$, $^{**}P < .01$, and $^{***}P < .001$ vs control.

24 hours (Fig. 3B). L-Glucose, 3-OMG, and mannitol at a concentration of 25 mmol/L did not cause CEL formation after 24-hour incubation of VSMCs (data not shown).

3.3. Effect of different MG precursors on the generation of nitric oxide and oxidized DCF (peroxynitrite)

Aminoacetone, fructose, and D-glucose all increased NO production, measured as the stable products nitrite + nitrate (Fig. 4A), and oxidized DCF levels (an indicator of peroxynitrite formation) (Fig. 4B) in VSMCs when 25 mmol/L of each substrate was incubated for 3 hours. Aminoacetone was the most potent inducer of NO and oxidized DCF production. No significant increases in nitrite + nitrate levels and oxidized DCF level were observed when

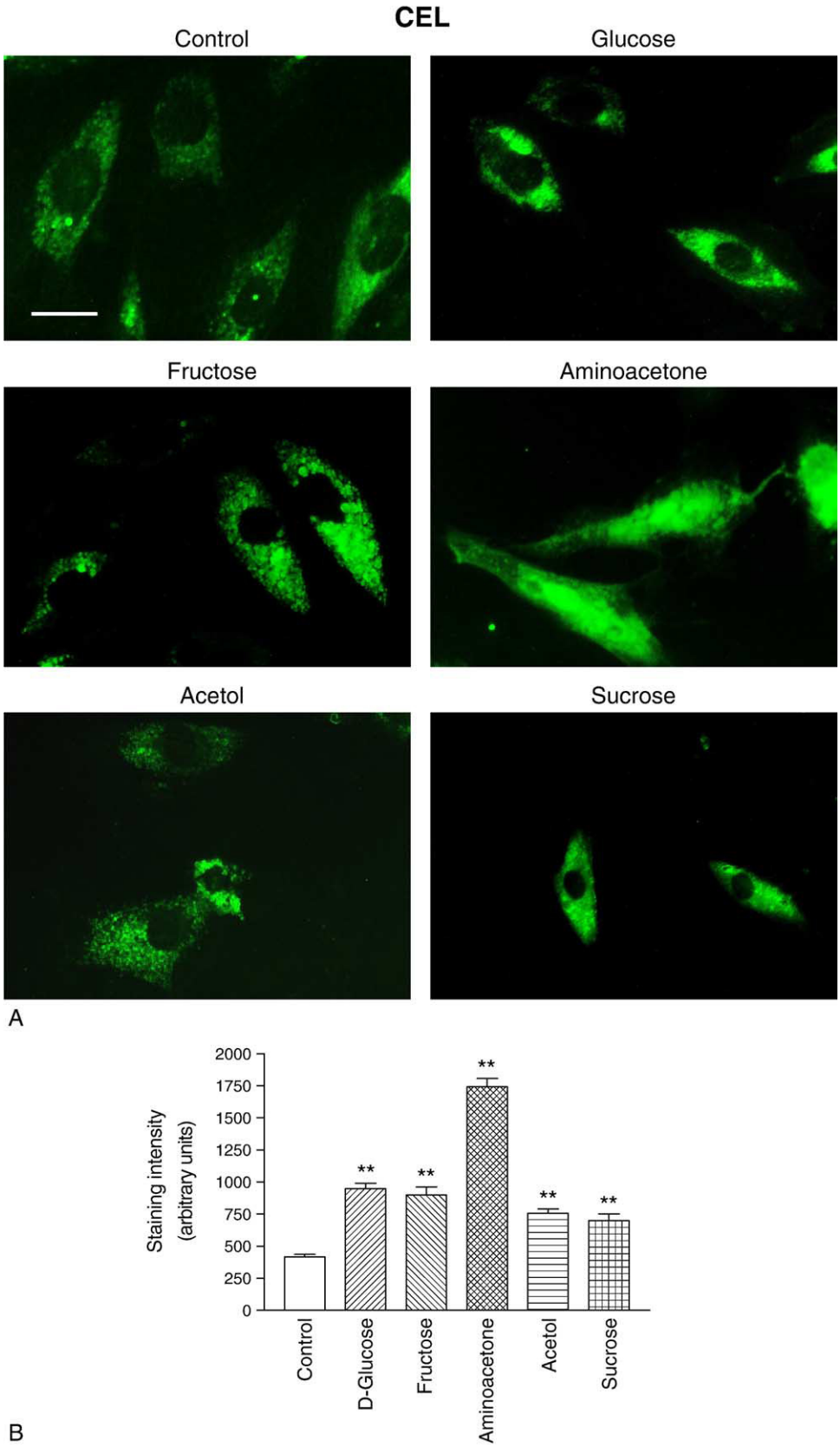


Fig. 3. Detection of MG-induced AGE CEL in cultured rat aortic smooth muscle cells (A-10) after incubation with different substrates (25 mmol/L each) for 24 hours. A, Immunocytochemistry was performed on fixed cells with specific CEL antibody and fluorescein isothiocynate–conjugated secondary antibody. Scale bar, 10 μm. B, Staining intensity was quantified using the Metamorph imaging program (Molecular Devices). $^{**}P < .01$ vs control.

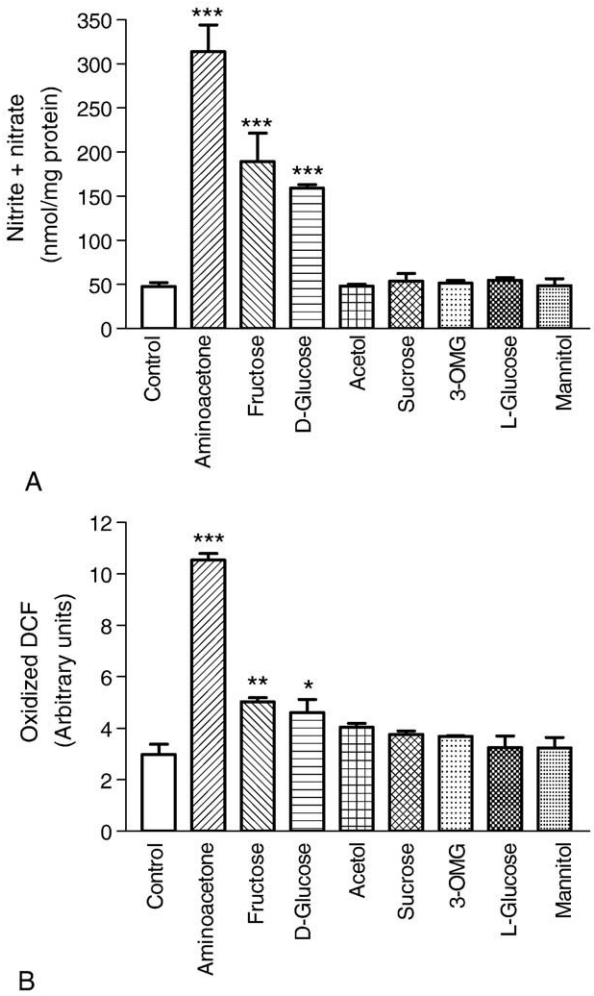


Fig. 4. Effect of different substrates on the production of nitrite + nitrate (nitric oxide) (A) and oxidized DCF (B) in cultured rat aortic smooth muscle cells (A-10). The production of nitrite + nitrate and oxidized DCF was determined after A-10 cells were incubated for 3 hours with different substrates (25 mmol/L each). n = 3 to 6 for each group. *$P < .05$, **$P < .01$, and ***$P < .001$ vs control.

VSMCs were treated for 3 hours with 25 mmol/L each of sucrose, acetol, L-glucose, 3-OMG, or mannitol (Fig. 4A, B).

3.4. Effect of MG precursors on iNOS expression

The staining intensity for iNOS was increased in VSMCs after incubation with aminoacetone, fructose, and D-glucose, each at 25 mmol/L for 3 hours (Fig. 5A). The greatest increase was induced by aminoacetone, followed by fructose and D-glucose (Fig. 5B). Acetol, sucrose, L-glucose, 3-OMG, and mannitol at a concentration of 25 mmol/L did not cause iNOS induction after 3-hour incubation of VSMCs (data not shown).

The fold increase in MG, NO, and oxidized DCF production has been shown in Fig. 6. This representation shows correlation of the magnitude of increase in nitrite + nitrate levels with the increase in MG levels after incubation with aminoacetone, fructose, and D-glucose. In case of oxidized DCF, the ratio of oxidized DCF/MG showed correlation with the increase in MG levels obtained with aminoacetone, fructose, and D-glucose (Fig. 6).

4. Discussion

We report major differences in MG produced in rat aortic smooth muscle cells incubated with different metabolic precursors derived from carbohydrates, proteins, and fatty acids. D-Glucose, fructose, and aminoacetone induced concentration-dependent increase of MG. Aminoacetone, an intermediary product of L-threonine metabolism, produced the greatest amount of MG with a 7-fold increase above the baseline value (Fig. 6). Fructose and D-glucose at an equimolar concentration of 25 mmol/L produced 3.9- and 3.5-fold increase in MG levels, respectively. Acetol and sucrose also produced significant MG, whereas L-glucose, 3-OMG, and mannitol did not (Figs. 2B and 6). Importantly, aminoacetone, even at 5 mmol/L, produced a significant increase in MG above the baseline levels, whereas 5 mmol/L D-glucose and fructose did not (Fig. 2A).

Aminoacetone, fructose, D-glucose, acetol, and sucrose also caused MG-induced AGE, CEL formation after 24-hour incubation with VSMCs. Methylglyoxal reacts with cysteine, arginine, and lysine residues of different proteins in a reversible or irreversible manner to form different end products. N^{ϵ}-carboxyethyl-lysine, which is formed when MG reacts with lysine residues of proteins [9], has been detected in human lens proteins at a concentration similar to that of CML [9], which is one of the most frequently found MG-derived AGEs [8]. N^{ϵ}-carboxyethyl-lysine increased with age in parallel with the concentration of CML and was formed in highest yields during the reaction of MG with lysine and protein, and the authors suggested that CEL levels can provide an index of glyoxal and MG concentrations in tissues [9]. In a recent study, levels of MG-derived hydroimidazolone correlated with the levels of CML [24], which in turn correlates with CEL formation [9]. These AGEs can be detected immunohistochemically in tissues [8,9,14].

Aminoacetone, fructose, and D-glucose also caused significant increase in NO and oxidized DCF formation (an indicator of peroxynitrite formation) and induced iNOS after 3-hour incubation with VSMCs, whereas acetol, sucrose, L-glucose, 3-OMG, and mannitol had no effect on NO and oxidized DCF formation and iNOS expression. It should be noted that, although the probe CM-H2DCFDA has high reactivity with peroxynitrite and its products CO_3^- and NO_2 [22], it is not entirely specific for peroxynitrite. As mentioned in Methods, this probe also has low reactivity for hydrogen peroxide and even lower for superoxide [22]. Previously, we have shown that exogenous MG can induce iNOS and increase superoxide and peroxynitrite in VSMCs [17,18]. In this study, the levels of MG correlate with nitrite + nitrate formed from different substrates (Fig. 6) and suggest a direct link between the amount of MG formed endogenously and the magnitude of NO formation. One possible source of NO

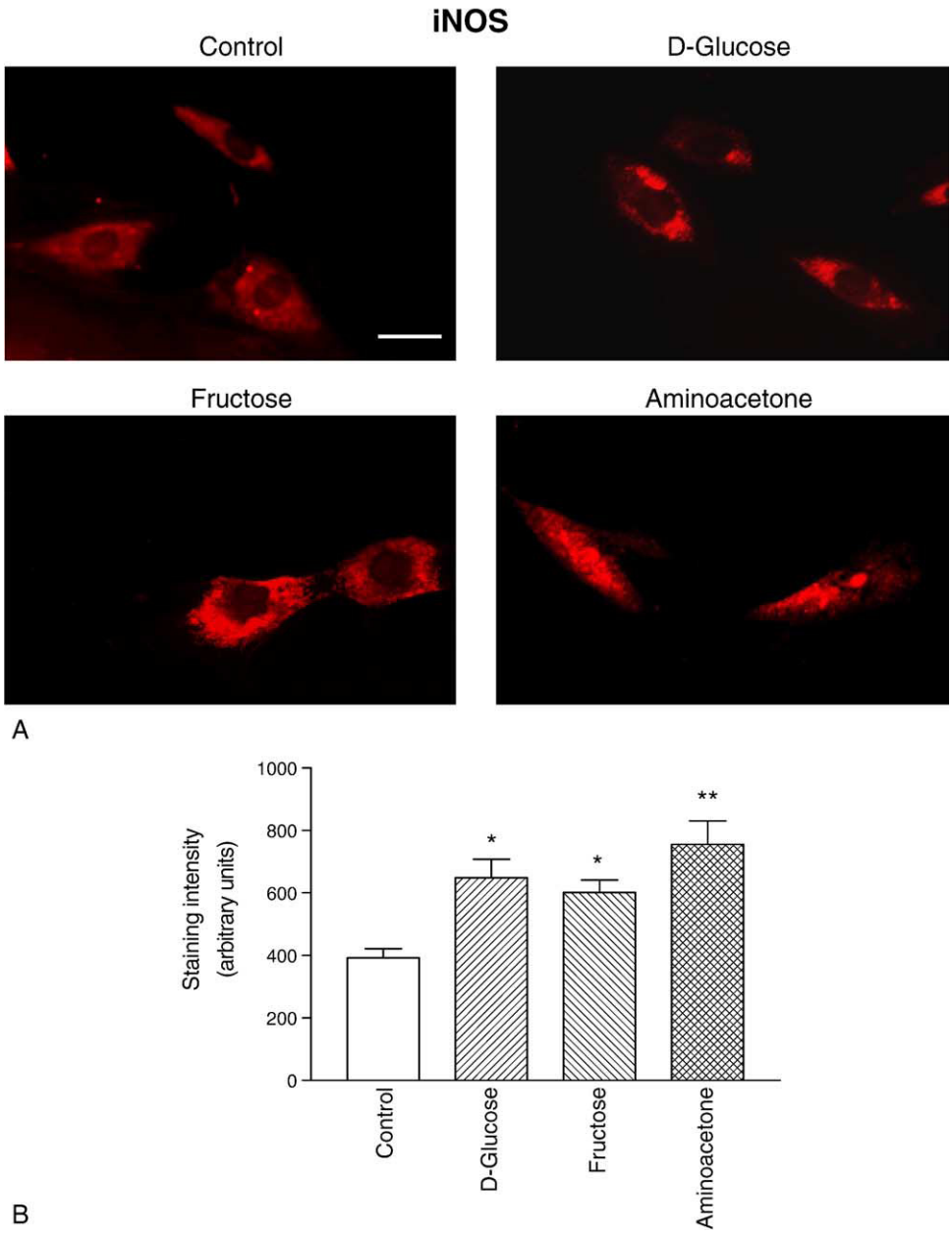


Fig. 5. Detection of iNOS in cultured rat aortic smooth muscle cells (A-10) after incubation with different substrates (25 mmol/L each) for 3 hours. A, Immunocytochemistry was performed on fixed cells with specific iNOS antibody and Texas red–conjugated secondary antibody. Scale bar, 10 μm. B, Staining intensity was quantified using the Metamorph imaging program (Molecular Devices). *$P < .05$ and **$P < .01$ vs control.

could be the increased levels of iNOS induced by aminoacetone, fructose, and D-glucose as shown here (Fig. 5A, B) [18-20]. Increased levels of iNOS are known to be associated with states of inflammation and immune reactions [25]. Thus, MG can trigger an inflammatory reaction partly through induction of iNOS. Increased NO in turn can lead to increased formation of peroxynitrite, which can nitrosylate proteins and cause cellular damage [19].

Methylglyoxal is detoxified via the glyoxalase system to form the inert D-lactate [3]. Activity of the glyoxalase system is dependent on adequate levels of reduced glutathione (GSH) [3]. Oxidative stress and hyperglycemia both lead to depletion of GSH [14,16,26]. Moreover, MG has been shown to inactivate glutathione peroxidase and glutathione reductase [16,26], which is required to recycle the oxidized glutathione back to GSH. This will impair the detoxification of MG and increase its half-life.

The high levels of MG, NO, and oxidized DCF (peroxynitrite) produced by aminoacetone may assume importance in conditions of increased amino acid metabolism, in particular increased L-threonine metabolism in people who are taking high amounts of protein such as athletes and body builders [27] or in conditions of increased SSAO level such as hypertension and diabetes

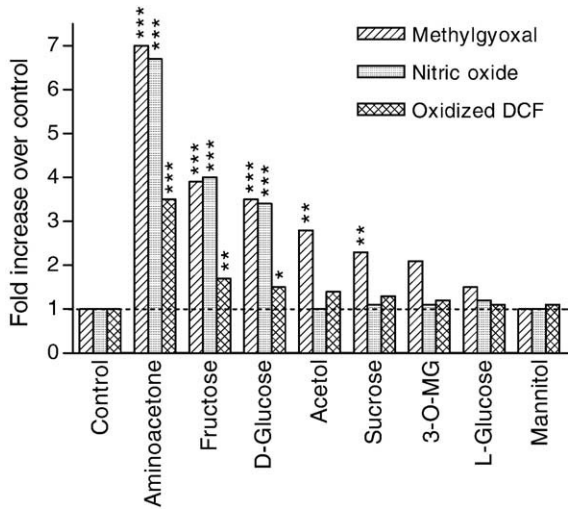


Fig. 6. Fold increase in MG, nitric oxide (nitrite + nitrate), and oxidized DCF levels above basal values after incubation of cultured rat aortic smooth muscle cells for 3 hours with different substrates (25 mmol/L each). *$P < .05$, **$P < .01$, and ***$P < .001$ vs control.

[4,6]. L-Threonine is metabolized to aminoacetone, which can be deaminated to MG by SSAO, especially if the level of the antioxidant GSH is compromised as in cases of increased oxidative stress [4,10,12,16]. Aminoacetone can be detected in the urine of normal people as well as patients with acute intermittent porphyria [28]. However, it must be admitted that plasma levels of aminoacetone in the range of 25 mmol/L that have been used in our in vitro study have not been reported in the literature. We could not find any literature reports on the intracellular levels of aminoacetone, thus limiting the significance of our findings regarding aminoacetone. Even so, the potential of aminoacetone to form more MG and oxidative stress on a molar basis is a significant finding of our study.

Fructose produced equivalent amounts of MG, nitrite + nitrate, and oxidized DCF (peroxynitrite) as D-glucose (Fig. 6). In healthy people, the plasma concentrations of fructose are low, around 0.13 mmol/L, compared with that of D-glucose, around 5 mmol/L; and it may not contribute to extracellular glycation as much as glucose [29]. However, intracellular fructose is elevated in a number of tissues of diabetic patients in which the polyol pathway is active and the concentrations of intracellular fructose and glucose are similar [30]. Plasma levels of fructose also increase in response to high fructose intake [29]. No dietary fructose is needed because required amounts are modest and can be easily produced endogenously from glucose through the aldose reductase pathway [30]. Most fructose is consumed as refined sugar, such as sucrose, and through fruits and vegetables. In most Western societies, the main source of fructose is high-fructose corn syrup that is added to many industrial food products including ketchup and bread [29]; and the daily intake of fructose may be as high as 100 g [31]. The glycemic index (GI) reflects the magnitude of increase in blood glucose levels after consuming a given carbohydrate. A GI of 55 or less is classified as low; between 56 and 69, medium; and more than 70, high [32]. The GI for a 50-g dose of fructose varies between 12 and 24 depending on the manufacturer [32]. This is much lower than a GI between 85 and 111 for a 50-g dose of glucose. The GI for 50 g sucrose is between 58 and 65 [32]. Because of the low GI of fructose, diabetic patients are advised to eat lots of fresh fruits and vegetables [33,34]. This advice needs to be carefully reevaluated in light of the equipotency of fructose, compared with D-glucose, in producing increased amounts of MG, NO, and peroxynitrite (Fig. 6). Interestingly, in one study, it was reported that, in a population of vegetarians and omnivores, plasma AGEs were higher [35]. This was linked to a higher intake of fructose derived from a higher consumption of vegetables and fruit. Thus, it is likely that the increased formation of MG from fructose, as shown here, may substantially contribute to the formation of intracellular AGEs, damage to cellular proteins, and vascular complications [36,37].

As mentioned earlier, acetol is a precursor for MG formation. An equimolar concentration of acetol produced a 2.8-fold increase in MG levels compared with the 3.5-fold produced by D-glucose (Fig. 6). Acetone, which is converted to acetol, is one of the ketone bodies; and increased plasma levels of acetone are encountered in situations of abnormally high lipolysis as in diabetic ketoacidosis [38], as well as during extended periods of fasting where there is increased fatty acid metabolism and with the ketogenic diet in children with epilepsy [39]. The ketogenic diet, composed of 80% to 90% fat and 10% to 20% carbohydrates and protein, is used for the treatment of drug-resistant seizures in children [39]. Mean serum acetone levels were 4 mmol/L in children on the ketogenic diet and up to 8 mmol/L in some of them [40]. Acetone levels up to 8.9 mmol/L were found in patients with diabetic ketoacidosis [38]. Intracellular acetone/acetol levels are likely to be much higher than these values. Thus, there is a potential for increase in MG levels in these situations; and indeed, increased acetone (6.12-fold) and MG levels (1.67-fold) have been reported in subjects taking the Atkins diet, which has a higher percentage of fat [41].

Sucrose is the common sugar from sugar beets or sugarcane [29]. Significant amounts are consumed with many sweet fruits. The sucrose in peaches may constitute as much as half of their dry weight. Sucrose is converted by the intestinal epithelium into glucose and fructose for absorption [29]. Sucrose produced a relatively smaller increase in MG and CEL compared with D-glucose, and no significant increase in nitrite + nitrate or oxidized DCF (peroxynitrite). The result seems to indicate that sucrose is not quickly converted to glucose and fructose in a short period in the VSMCs.

L-Glucose is the metabolically inert isomer of D-glucose [29], whereas 3-OMG is a nonmetabolizable glucose analogue that is not phosphorylated by hexokinase. 3-*O*-methylglucose is used as a marker to assess glucose transport

by evaluating its uptake within various cells and organ systems [42]. Mannitol is commonly used for its osmotic effect [43] and is not likely to be metabolized. As anticipated, L-glucose, 3-OMG, and mannitol did not increase MG, CEL, nitrite + nitrate, and oxidized DCF (peroxynitrite) above the basal levels (Fig. 6). Thus, MG production is restricted to specific substrates.

The choice of 25-mmol/L concentration is based on the observation that plasma glucose level in diabetic patients or streptozotocin-induced diabetic rats [44] can reach this high level; and as such, this concentration level can be used as a benchmark to compare the capabilities of other metabolic precursors on MG production.

In conclusion, the amount of MG formed in VSMCs depends a great deal on the nature of the substrate. The important finding of our study is that aminoacetone has the potential to form significant amounts of MG, CEL, NO, and oxidized DCF (peroxynitrite) even at low concentrations. Because aminoacetone is an intermediate of protein metabolism, the production of MG and peroxynitrite under conditions of increased protein catabolism is worthy of further investigation. Another key finding is that fructose, despite its low GI value compared with D-glucose, can form equal amounts of MG and CEL and induce oxidative stress as D-glucose. Increased fructose consumption as seen in Western diets may be a health concern. The advice given to diabetic patients to eat lots of fruits that contain fructose needs to be carefully reevaluated. Because currently the main tendency is to focus on glucose in terms of increased MG, AGEs, and oxidative stress in conditions such as diabetes, it is important to consider the contribution of other substrates such as fructose, acetol, and aminoacetone to MG, AGEs, and oxidative stress load. This is despite their lower concentrations and apparently no correlation to high glucose levels. Our findings also bear relevance to situations where the metabolic pathways are altered and the intracellular concentrations of metabolites such as fructose, aminoacetone, and acetone/acetol may reach high levels.

Acknowledgment

This work was supported by an operating grant from the Canadian Institutes of Health Research (CIHR, MOP-68938) and the Heart and Stroke Foundation of Canada to L Wu. L Wu is supported by a New Investigator Award from CIHR. A Dhar is supported by a Gasotransmitter REsearch And Training program grant provided by CIHR/Heart and Stroke Foundation of Canada.

References

[1] Desai K, Wu L. Methylglyoxal and advanced glycation end products: new therapeutic horizons? Recent Pat Cardiovas Drug Res 2007;2:89-99.

[2] Thornalley PJ. Pharmacology of methylglyoxal: formation, modification of proteins and nucleic acids, and enzymatic detoxification—a role in pathogenesis and antiproliferative chemotherapy. Gen Pharmacol 1996;27:565-73.

[3] Kalapos MP. Methylglyoxal in living organisms: chemistry, biochemistry, toxicology and biological implications. Toxicol Lett 1999;110:145-75.

[4] Deng Y, Boomsma F, Yu PH. Deamination of methylamine and aminoacetone increases aldehydes and oxidative stress in rats. Life Sci 1998;63:2049-58.

[5] Casazza JP, Felver ME, Veech RL. The metabolism of acetone in rat. J Biol Chem 1984;259:231-6.

[6] Yu PH, Wright S, Fan EH, Lun ZR, Gubisne-Harberle D. Physiological and pathological implications of semicarbazide-sensitive amine oxidase. Biochim Biophys Acta 2003;1647:193-9.

[7] Shipanova IN, Glomb MA, Nagaraj RH. Protein modification by methylglyoxal: chemical nature and synthetic mechanism of a major fluorescent adduct. Arch Biochem Biophys 1997;344:29-36.

[8] Reddy S, Bichler J, Wells-Knecht KJ, Thorpe SR, Baynes JW. N epsilon-(carboxymethyl)lysine is a dominant advanced glycation end product (AGE) antigen in tissue proteins. Biochemistry 1995;34:10872-8.

[9] Ahmed MU, Brinkmann Frye E, Degenhardt TP, Thorpe SR, Baynes JW. N-epsilon-(carboxyethyl)lysine, a product of the chemical modification of proteins by methylglyoxal, increases with age in human lens proteins. Biochem J 1997;324:565-70.

[10] Vlassara H, Bucala R, Striker L. Pathogenic effects of advanced glycosylation: biochemical, biologic, and clinical implications for diabetes and aging. Lab Invest 1994;70:138-51.

[11] Wang H, Meng QH, Gordon JR, Khandwala H, Wu L. Proinflammatory and proapoptotic effects of methylglyoxal on neutrophils from patients with type 2 diabetes mellitus. Clin Biochem 2007;40:1232-9.

[12] McLellan AC, Thornalley PJ, Benn J, Sonksen PH. Glyoxalase system in clinical diabetes mellitus and correlation with diabetic complications. Clin Sci (Lond) 1994;87:21-9.

[13] Jia X, Olson DJ, Ross AR, Wu L. Structural and functional changes in human insulin induced by methylglyoxal. FASEB J 2006;20:1555-7.

[14] Wang X, Desai K, Chang T, Wu L. Vascular methylglyoxal metabolism and the development of hypertension. J Hypertens 2005;23:1565-73.

[15] Wang X, Desai K, Wu L. Attenuation of hypertension development by aminoguanidine in spontaneously hypertensive rats: role of methylglyoxal. Am J Hypertens 2007;20:629-36.

[16] Wu L, Juurlink BH. Increased methylglyoxal and oxidative stress in hypertensive rat vascular smooth muscle cells. Hypertension 2002;39:809-14.

[17] Chang T, Wang R, Wu L. Methylglyoxal-induced nitric oxide and peroxynitrite production in vascular smooth muscle cells. Free Radic Biol Med 2005;38:286-93.

[18] Wang H, Meng QH, Chang T, Wu L. Fructose-induced peroxynitrite production is mediated by methylglyoxal in vascular smooth muscle cells. Life Sci 2006;79:2448-54.

[19] Pacher P, Beckman JS, Liaudet L. Nitric oxide and peroxynitrite in health and disease. Physiol Rev 2007;87:315-424.

[20] Hoare GS, Marczin N, Chester AH, Yacoub MH. Role of oxidant stress in cytokine-induced activation of NF-kappaB in human aortic smooth muscle cells. Am J Physiol 1999;277:H1975-84.

[21] Bucala R, Model P, Cerami A. Modification of DNA by reducing sugars: a possible mechanism for nucleic acid aging and age-related dysfunction in gene expression. Proc Natl Acad Sci U S A 1984;81:105-9.

[22] Wardman P. Fluorescent and luminescent probes for measurement of oxidative and nitrosative species in cells and tissues: progress, pitfalls and prospects. Free Radic Biol Med 2007;43:995-1022.

[23] Kazachmov M, Yu P. A novel HPLC procedure for detection and quantification of aminoacetone, a precursor of methylglyoxal, in biological samples. J Chromatogr B Analyt Technol Biomed Life Sci 2005;824:116-22.

[24] Kilhovd B, Giardino I, Torjesen PA, Birkeland KI, Berg TJ, Thornalley PJ, et al. Increased serum levels of the specific AGE-compound

methylglyoxal-derived hydroimidazolone in patients with type 2 diabetes. Metabolism 2003;52:163-7.
[25] Kaneki M, Shimizu N, Yamada D, Chag K. Nitrosative stress and pathogenesis of insulin resistance. Antioxid Redox Signal 2007;9: 319-29.
[26] Park YS, Koh YH, Takahashi M, Miyamoto Y, Suzuki K, Dohmae N, et al. Identification of the binding site of methylglyoxal on glutathione peroxidase: methylglyoxal inhibits glutathione peroxidase activity via binding to glutathione binding sites Arg 184 and 185. Free Radic Res 2003;37:205-11.
[27] Brandle E, Sieberth HG, Hautmann RE. Effect of chronic dietary protein intake on the renal function in healthy subjects. Eur J Clin Nutr 1996;50:734-40.
[28] Tschudy DP, Welland FH, Collinsunter G. Aminoacetone in acute intermittent porphyria. Lancet 1963;186:660-1.
[29] Kohlmeier M. Carbohydrates, alcohols, and organic acids. Fructose. Oligosaccharides (sucrose). In: SL Taylor, Series Editor. Nutrient metabolism (Food science and technology. International series). San Diego (Calif): Academic Press, Elsevier Ltd. 2006, Ch. 7. p. 189, 210-14.
[30] Tomlinson DR, Willars GB, Carrington AL. Aldose reductase inhibitors and diabetic complications. Pharmacol Ther 1992;54: 151-94.
[31] Ruxton CH, Garceau FJ, Cottrell RC. Guidelines for sugar consumption in Europe: is a quantitative approach justified? Eur J Clin Nutr 1999;53:503-13.
[32] Glycemic index. Available on-line at: http://www.glycemicindex.com/
[33] Diabetes nutrition: general dietary guidelines. Available on-line at: http://www.diabetes.co.uk/nutrition.html
[34] Making healthy food choices. Available on-line at: http://www.diabetes.org/nutrition-and-recipes/nutrition/healthyfoodchoices.jsp
[35] Krajcovicova-Kudlackova M, Sebekova K, Schinzel R, Klvanova J. Advanced glycation end products and nutrition. Physiol Res 2002;51: 313-6.
[36] Mikulikova K, Eckhardt A, Kunes J, Zicha J, Miksik I. Advanced glycation end-product pentosidine accumulates in various tissues of rats with high fructose intake. Physiol Res 2008;57:89-94.
[37] Tokita Y, Hirayama Y, Sekikawa A, Kotake H, Toyota T, Miyazawa T, et al. Fructose ingestion enhances atherosclerosis and deposition of advanced glycated end-products in cholesterol-fed rabbits. J Atheroscler Thromb 2005;12:260-7.
[38] Owen OE, Trapp VE, Skutches CL, Mozzoli MA, Hoeldtke RD, Boden G, et al. Acetone metabolism during diabetic ketoacidosis. Diabetes 1982;31:242-8.
[39] Gasior M, French A, Joy MT, Tang RS, Hartman AL, Rogawski MA. The anticonvulsant activity of acetone, the major ketone body in the ketogenic diet, is not dependent on its metabolites acetol, 1,2-propanediol, methylglyoxal, or pyruvic acid. Epilepsia 2007;48:793-800.
[40] Musa-Veloso K, Likhodii SS, Rarama E, Benoit S, Liu YM, Chartrand D, et al. Breath acetone predicts plasma ketone bodies in children with epilepsy on a ketogenic diet. Nutrition 2006;22:1-8.
[41] Beisswenger BGK, Delucia EM, Lapoint N, Sanford RJ, Beisswenger PJ. Ketosis leads to increased methylglyoxal production on the Atkins diet. Ann N Y Acad Sci 2005;1043:201-10.
[42] Dringen R, Hamprecht B. Inhibition by 2-deoxyglucose and 1,5-gluconolactone of glycogen mobilization in astroglia-rich primary cultures. J Neurochem 1993;60:1498-504.
[43] Paczynski RP. Osmotherapy. Basic concepts and controversies. Crit Care Clin 1997;13:105-29.
[44] Cheng JT, Liu IM, Chi TC, Tzeng TF, Lu FH, Chang CJ. Plasma glucose-lowering effect of tramadol in streptozotocin-induced diabetic rats. Diabetes 2001;50:2815-21.