Oxidative stress, protein glycation and nutrition – interactions relevant to health and disease throughout the lifecycle.

Mr Vlassopoulos Antonis*, Professor Lean Michael EJ and Dr Combet Emilie

Human Nutrition, School of Medicine, College of Medical, Veterinary & Life Sciences, University of Glasgow , Glasgow, G31 2ER, UK

*Corresponding Author: Tel +44 (0) 141 201 8605, Fax: +44(0)141 201 4844, E-mail: a.vlassopoulos.1@research.gla.ac.uk

Abstract

Protein glycation has been studied for over a century now and plays an important role in disease pathogenesis throughout the lifecycle. Strongly related to diabetic complications, glycation of haemoglobin has become the gold standard method for diabetes diagnosis and monitoring. It is however attracting attention in normoglycaemia as well lately. Longitudinal studies increasingly suggest a positive relationship between glycation and the risk of chronic diseases in normoglycaemic individuals, but the mechanisms behind this association remain unclear. The interaction between glycation and oxidative stress may be particularly relevant in the normoglycaemic context, as suggested by recent epidemiological and *in-vitro* evidence. In that context nutritional and lifestyle factors with an influence on redox status, such as smoking, fruit and vegetable and antioxidants consumption, may have the capacity to promote or inhibit glycation. However, experimental data from controlled trials are lacking the quality and rigor needed to reach firm conclusions. In this review, we discuss the importance of glycation for health through the lifecycle and focus on the importance of oxidative stress as a driver for glycation. The importance of nutrition to modulate glycation is discussed, based on the evidence available and recommendations towards higher quality future research are made.

Key-Words: glycation, oxidative stress, antioxidant, nutrition, diabetes mellitus, chronic disease, polyphenols, RAGE

The glycation reaction - historical background

Glycation, also referred to as non-enzymatic browning or the Maillard reaction, has attracted scientific interest for nearly a century. Initiated by the non-enzymatic condensation of a reducing sugar (like glucose) with a protein, glycation is one of the most important forms of protein damage/loss, relevant to both medicine and food science. Named after the pioneer in the field, the Maillard reactions were described in 1912[1] and systematically presented for the first time by John E. Hodge in 1955[2]. During the early years, glycation was studied in the context of food science, food processing and hence relative to health via nutritional intake. In 1977, a fraction of haemoglobin, HbA1c, was identified as a ketoamine (glycation product) and the concept of *in-vivo* protein glycation gradually became mainstream[3-5]. HbA1c was proposed as a useful biomarker for diabetes monitoring[3; 4], and endogenously produced Advanced Glycation Endproducts (AGEs) have since attracted further scientific attention, beyond food chemistry, from fields including medical biochemistry and pathology.

The importance of glycation for health

Glycation and the AGE-RAGE axis

The study of the role played by glycation in disease pathogenesis originally relied on measuring fructosamine levels in biological fluids, combined with the characterisation of endogenous AGEs in the circulation and tissues[6; 7]. These measurements were related to glycaemia and the topic very much focused on diabetes[3-5].

In hyperglycaemia (post-prandially or in non-controlled diabetes) and to a lesser extent in normoglycaemia, both circulatory proteins and proteins of the endothelium are exposed to (excess) glucose, leading to the slow formation of AGEs[8-11]. During that process, glycation adducts are created on the protein molecule, as a function of glucose levels. Accumulation of glycation adducts on the protein promotes excessive cross-linking with other protein molecules, which, in the case of collagen for example, would inhibit the formation of an ordered and functional polymeric complex. Such changes could lead to the formation of a thick vascular wall with i) reduced elasticity and ii) a high affinity of collagen to bind other circulating proteins like IgG, albumin and lipoproteins like LDL[12-21]. In turn, the immobilisation of proteins on the vascular wall will promote further glycation and cross-linking and will act as a signal for chemo-attraction of macrophages and monocytes, promoting inflammation and ‘foam’ cell formation in the endothelium[22-24].

The discovery that AGEs can bind on cellular receptors and alter intracellular events was a breakthrough, linking glycation to signalling[25]. Receptors like AGE-R1, AGE-R2, AGE-R3, MSRII, CD36, LOX-1 and the Receptor for AGEs (RAGE), the most characterised receptor[26], are multi-ligand cell-surface immunoglobulins, with the ability to initiate injury-like intracellular events, mainly expression of genes related with inflammation and oxidative stress[27-29]. Upon activation of RAGE, intracellular ROS levels are increased through up-regulation of NAD(P)H oxidase expression. This in turns leads to the activation of the Ras-MAP kinase pathway, ultimately up-regulating NFκB and the production of inflammatory molecules (including TNF-a, VCAM-1, I-CAM1 and IL-1beta). The up-regulation of NFκB also initiates a positive feedback loop that sensitises the cell (and hence the tissue) to AGEs by promoting RAGE production[24].

Together accumulation of AGEs in tissues and AGE-RAGE interactions are the two main pathways of glycation involvement in disease pathogenesis. These two pathways are often acting simultaneously and their individual effects are hard to distinguish; hence they are commonly presented in the same context when discussing glycation related pathophysiology[12; 30-34].

Glycation and health throughout the lifecycle

Glycation is relevant to all stages in the lifecycle, including conception and early gestation. The reproductive tract is a known site for AGEs accumulation both in men[35] and women[36]. AGEs accumulation is followed by changes in the distribution of RAGE in reproductive tissues[37], and sRAGE (the soluble isoform of RAGE) in seminal/follicular fluid[38; 39], which may lead to lower sperm quality[38], lower likelihood of success following assisted reproduction[40; 41] and reduced embryonal quality and development[39; 41; 42]. During the course of pregnancy, activation of the AGE-RAGE axis may be involved in the pathogenesis of preeclampsia[43-45]. So far evidence on the involvement of AGEs and/or RAGE in fetal development are limited and based on animal studies. For example a study on transgenic mice showed that overexpression of RAGE was associated with impairments in alveolar morphogenesis. The degree of RAGE overexpression was related to the magnitude of the abnormality with homozygous mice having histological changes similar to human bronchopulmonary dysplasia. The study also found that this early life changes could lead to increased risk of 'destructive' emphysema[46]. Glycation has also been proposed as a mechanism of ageing[47; 48]. Evidence from animal models suggest that a diet low in AGEs (50% reduction in AGEs intake) was associated with amelioration of insulin resistance, lower AGEs accumulation (both indications of the ageing process) and ultimately increased lifespan compared to the controls[49]. Similarly, mice on caloric restriction, a popular model of lifespan expansion in animal models, have lower levels of collagen cross-linking and lower levels of lens cataract, suggesting lower AGEs accumulation in the vitreous and the extracellular matrix[50; 51] as well as in the brain[52].

In fact, mice fed high AGEs diets while on caloric restriction did not show any increase in their lifespan and the authors of the report suggested that lower AGEs intake may be one of the mechanisms behind the caloric restriction model[49; 53]. An interesting observation linking the effect of AGEs in ageing and as early in life as in conception comes from a study showing the active involvement of AGEs accumulation in ovarian ageing and ovarian function in human subjects[54].

HbA1c and risk of chronic diseases

Even though the exact mechanisms of disease pathogenesis remain elusive, extensive evidence is available to associate glycation with disease risk. Glycation has a particular relevance for age-related diseases, including Alzheimer's disease[55; 56], skin ageing[48] and cataract[47]. These conditions are characterised by increased, possibly lifelong, deposition of AGEs in the affected tissue[57-59].

As *in-vivo* glycation is believed to be mainly driven by plasma glucose concentrations, the most established relationship is between glycation and diabetes. HbA1c is the gold standard method for diabetes diagnosis and monitoring[60]. According to the American Diabetes Association, individuals with HbA1c levels between 5.7-6.5 % are considered at high risk of developing diabetes. Those with HbA1c>6.5% are classified as having diabetes[61]. Among patients with diabetes, higher HbA1c levels are associated with increased risk of retinopathy[62-66], neuropathy[67] and nephropathy[66].

Glycation has recently attracted attention as a risk factor for normoglycaemic individuals. For the purpose of this paper, we conducted a systematic literature search to identify studies documenting the effect of increased glycation on the risk of non-communicable chronic diseases in normoglycaemic subjects. We identified 15 reports from 8 studies (European Prospective Investigation into Cancer and Nutrition-EPIC[68; 69], Atherosclerosis Risk in Communities study-ARIC[70-73], Australian Diabetes, Obesity and Lifestlyle study-AusDiab[74], the Hoorn Study[75; 76], Framingham Offspring[77], Rancho Bernardo[78], Women's Health Study-WHS[79-81] and National Survey of Cardiovascular Disorders 1990-NIPPON DATA90[82]) analysing data from a total of over 63,000 participants, followed-up for 4-15 years. The outcomes of interest were diabetes risk, cardiovascular disease (CVD), ischemic heart disease, stroke, coronary heart disease (CHD) and all-cause and CVD mortality. Two reports focused on the association between glycation and cancer risk, especially colorectal[69] and breast cancer[80]. Overall, the studies showed a positive relationship between higher HbA1c and the risk of stroke and/or CVD and/or mortality ranging between 18-55% higher risks per 1% increase in HbA1c[68-74; 82] . As far as cancer incidence is

concerned, the results are still inconclusive. Data from the EPIC cohort suggest a 33% increase in the incidence of colorectal cancer per every 1% increase in HbA1c[69], but an analysis of the WHS data did not find any association between HbA1c and breast cancer risk[80]. As the two cancer types differ significantly in aetiology, colorectal cancer has a strong dietary link[83] while breast cancer is mainly of genetic aetiology[84]; more research is needed before any conclusion is reached.

Oxidative stress and protein glycation in normoglycaemia

As observed by Selvin et al[70], fasting glucose may fail to explain the positive relationship between HbA1c and CVD and/or mortality. Correction for classical risk factors (including smoking, dyslipidaemia, inflammation) explain the relationship better[75; 76; 79; 81], suggesting that a shared mechanism may drive the increase in HbA1c levels. Although indications and potential mechanisms are in place to suggest an active involvement of oxidative stress in protein glycation in normoglycaemia and hence the increase in the risk of chronic diseases, so far little evidence is available to support such a hypothesis.

In our previous work, we hypothesised that oxidative stress could be this shared mechanism, which acts as a glycation driver in normoglycaemia.

Using the Scottish Health Surveys (SHS) datasets 1993-2010, we have shown that, in individuals without diabetes and HbA1c levels lower than 6.5%, age-sex adjusted HbA1c levels are positively correlated with smoking status, an association seen even among ex-smokers who used to smoke regularly[85]. Smoking status was used as a proxy for oxidative stress and, in a similar way, fruit and vegetable intake was used as a proxy for antioxidant intake. Smoking was positively associated with HbA1c levels from as few as 10 cigaretes per day a finding consistent with previous reports [86; 87] (Figure1). The likelihood of having an HbA1c level within the prediabetes range (5.7-6.4%) was double among smokers compared to non- smokers; this was seen even with less than 10 cigarettes per day smoked. Interestingly, smoking cessation does not lead to complete reversal to the non-smoking state, as former smokers were found to have lower HbA1c levels than smokers but not as low as never smokers[86; 87]. In a linear regression model, smoking was associated with 0.08% higher HbA1c compared to no smoking, which is equal to 0.25 times the SD. As expected, vegetable intake had the opposite effect being associated with lower age-sex adjusted HbA1c levels with more portions consumed. In fact, for every extra 80g portion of vegetable consumed there was an associated 0.01% reduction in HbA1c.

The hypothesis that glycative and oxidative damage are closely related *in vivo* is supported by evidence showing that in purified plasma albumin, oxidative damage - measured as a reduction in free thiol groups - was positively related to glycative damage, measured as fructosamine and

carbonyl rate[88]. Moreover, Cys-34, a key site of oxidative damage in albumin *in vivo*[89], has also been suggested as a glycation site, especially from a-oxoaldehydes[90]. Since *in-vitro* models are often removed from physiologically-relevant reactions, it is important to setup mechanistic studies with adequate parameters. To test the hypothesis that, in normoglycaemia, oxidative stress promotes glycation, we carried out 4-week long albumin incubation studies (albumin has a half-life of 14-28 days). Glucose concentrations of 5 and 10 mM were employed to replicate normoglycaemia and (non-controlled) diabetes, respectively, while 20 mM and 30 mM glucose were used as positive controls (supraphysiological concentrations). There is no consensus on the plasma levels of hydrogen peroxide (from nearly 0 to 35 μM[91-93]), we used a low concentration of hydrogen peroxide (H_2O_2, 10 nM) to simulate physiologically relevant oxidative stress [94]. Co-incubation of albumin with glucose and physiological levels of H_2O_2 led to significantly higher glycation at all glucose levels tested, after 2 weeks and 4 weeks incubation, compared to glucose alone. At physiological glucose level (5mM), there was no significant glycation (versus negative control) in absence of H_2O_2 (Figure 2), indicating that oxidative stress plays an important in glycation in normoglycaemia. Physiologically, in the presence of oxidative stress, proteins can get quickly oxidised and remain in this form in circulation until they are degraded by proteases[95]. As extracellular/circulating proteins are more likely to get oxidised first before getting glycated, due to the relative speed of the reactions, the same experiments were repeated using pre-oxidised protein. The pre-oxidised BSA led to a higher production of fructosamine when incubated with glucose as compared to the native incubated BSA. Oxidative stress also drove glycation of human plasma proteins, in presence of 5 mM glucose.

Brought together, these results[85; 96] indicate the potential role for oxidative stress as a driver for glycation in normoglycaemic individuals. The increased levels of HbA1c seen in smokers and those consuming low amounts of fruit and vegetables could be partially due to their impaired redox status, as stipulated by the epidemiological data. This interaction between oxidative stress and glycation will be subtle but with potentially sizeable long term effects. Hence, dietary interventions aiming to restore the antioxidant/pro-oxidant balance in subjects at high risk of oxidative stress could be of value in chronic disease prevention.

Antiglycative capacity of antioxidants and polyphenols

In the search for compounds able to inhibit or slow the glycation reaction, antioxidants have attracted attention. The first AGE blocker identified is aminoguanidine[97]; a dicarbonyl scavenging agent that reduces AGE production by removing the oxidatively produced precursors, like a-

oxoaldehydes[98; 99]. Aminoguanidine, like other glycation inhibiting compounds aspirin and ibuprofen, has the capacity to scavenge free radicals and improve redox status, which may contribute to their antiglycative capacity[99-101].

The antiglycative capacity of antioxidant vitamins and polyphenols has also been investigated, with *in-vitro* studies showing some polyphenols and phenolic acids to be even more effective than aminoguanidine in inhibiting glycation[102-104]. Herb extracts and commonly consumed herbal preparations have been shown to inhibit glycation of albumin in experimental settings. Red wine, green tea, maté tea (Ilex paraguariensis)[105; 106], cinnamon, garlic[107] and other herbs used to prepare hot drinks or added during cooking are rich in a variety of micronutrients with anti-glycative effects[108; 109]. A recent review of the literature by Xie et al.[110] analysed results from 19 *in-vitro* trials and 11 animal studies and concluded that antiglycative capacity of polyphenols is linked to ring hydroxylation patterns. In this context, molecules with hydroxyl groups in the A and B rings (i.e. apigenin < luteolin, fisetin < quercetin, daidzein < genistein) those with multiple hydroxyl groups especially in the *ortho*- and *meta*- structure (i.e. phloridzin < sieboldin), the proanthocyanidin di/trimmers and the ellagitannins all showed increased antiglycative capacity. On the other hand, hydrogenation of the C2-C3 bond (i.e. eriodictyol < luteolin), methylation (i.e. diosmetin < luteolin) and the addition of rutinosides all decreased the antiglycative capacity[110]. The results of *in-vitro* studies are still heterogeneous and a thorough review of the glycation models and assays used would help to understand why translation of the findings to a physiological setting has not been forthcoming. Some of the reasons include use of high glucose or fructose concentrations, supraphysiological concentrations of polyphenols/phenolic acids, use of compounds with very limited bioavailabilty, and variability in the incubation period/temperature. Doses tested *in vitro* are, most of the times beyond concentration that could be reached via habitual consumption of phenolic-rich foodstuff. Most polyphenols are metabolised extensively in the gut and by the liver after ingestion, and have generally a low bioavailability[111; 112]. Therefore studies focusing on the systemic effects of the “parent” compounds, as found in foods, are likely to have low translational values. Phenolic acids, such as 3-hydroxyphenylacetic acid, 3,4-dihydroxyphenylacetic acid and caffeic acid, on the other hand, are formed after exposure to the gut microbiota, have a higher bioavailability than larger polyphenols and are more likely to exert systemic effects[111; 112].

Despite the extensive mechanistic evidence, epidemiological data on polyphenol consumption are scarce. Principal reasons include the difficulties and the biases associated with deriving polyphenol intake data from dietary records. The process involves the use of databases, such as PhenolExplorer[113] documenting the polyphenol content of foods[113; 114] and/or the analysis of Food Frequency Questionnaires to identify patterns of higher intake of polyphenol-rich foods. So

far, there are no reports addressing the relationship between polyphenol intake and glycation levels. The reports associating polyphenol intake with diabetes risk have so far reached contradictory conclusions[115-117]. Our own systematic review of the literature relating antioxidant intake with protein glycation in normoglycaemia showed that human trials with polyphenol rich supplements and foods are few and characterised by high heterogeneity, poor design and small samples size (in preparation). In the past 20 years, only 14 trials used polyphenols as a mean to reduce glycation in non-diabetic individuals, out of which two did not have any control group[118; 119]. Taken together, the results of these studies seem to suggest that polyphenol supplementation fails to improve glycation markers in non-diabetic individuals, although this conclusion is most likely to be a result of poor study design. In populations with established IGT, increased intake of polyphenols might be promising in reducing protein glycation[120; 121], but no hard conclusions can be made at this point. The bioactive molecules tested were diverse with no standardisation in dose. The majority of the studies had glycation as a secondary outcome, leading to low statistical power, and did not have sufficient duration to detect changes, if any were present.

Considerations for the future

Although the importance of glycation as a marker of disease pathogenesis outside of diabetes is becoming clearer, it is yet to be fully understood. More studies are required to describe the interactions between oxidative stress and glycation, especially in normoglycaemia. The importance of RAGE activation to signal intracellular events that promote dysfunction and the factors that determine the levels of sRAGE have not attracted the required attention.

As far as polyphenol and antioxidant trials are concerned, there is still much improvement to be done in terms of study design before conclusions can be reached. If the working hypothesis is that polyphenols will exert health benefits via their antioxidant capacity, then markers to document such improvements should be included and results on glycation markers, like HbA1c, should be discussed alongside oxidative stress improvements.

Sample size and targeting the correct population are two key aspect of study design to be considered. Polyphenol supplementation in a relatively healthy population is likely to have a subtle effect on health markers and hence studies with large sample sizes are likely to be required[122]. The majority of the studies to-date fall short of that sample size and are hence likely to be underpowered. As a result, we should be careful in concluding that polyphenol supplementation has no effect on glycation. The current literature may be just describing a lack of power to detect such an effect if any.

A good understanding of the supplement used, with data on bioavailability, composition and dose would allow for a more effective comparison of the studies. Also ensuring that the study duration is sufficient to detect changes in glycation markers is a vital improvement. Albumin has a half-life of 14-28 days while hemoglobin's half-life is 90 days; studies with duration shorter than the half-life of the target protein are unlikely to detect any changes in protein glycation. Also even though physical protein damage is the main pathway of glycation-related pathogenesis; RAGE activation, sRAGE levels and glycation related inflammation are also important pathways for the involvement of glycation in disease pathogenesis, but are so far understudied[123; 124].

Conclusion

Glycation is an important mechanism of end organ damage and disease pathogenesis affecting individuals throughout the lifecourse. With many target molecules and mechanisms of actions glycation and oxidative stress are increasingly recognised as of clinical importance not only in diabetes but in normoglycaemia as well. Epidemiological and *in-vitro* data so far are supporting the hypothesis that oxidative stress and its regulation with antioxidants is of importance in an attempt to inhibit glycation, especially in normoglycaemia. Although the importance of nutrition in glycation regulation is becoming more apparent, clinical trials with polyphenols so far lack the quality to form conclusive decisions. More large scale and high quality interventions are needed before recommendations can be made.

Acknowledgements

The authors have no conflicts of interest to declare. AV is in receipt of a scholarship from Yorkhill Children's Foundation. AV conducted the studies described and drafted the manuscript. EC and MEJL secured the funding, supervised the studies and contributed to the drafting of the manuscript.

References

1. Maillard L (1912) Action des acides amines sur les sucres: Formation des melanoidines par voie methodologique. *Comptes Rendus de l'Academie des Sciences* **156**, 148-149.
2. Hodge JE (1955) The Amadori rearrangement. *Advances in carbohydrate chemistry* **10**, 169-205.
3. Koenig RJ, Peterson CM, Jones RL *et al.* (1976) Correlation of glucose regulation and hemoglobin AIc in diabetes mellitus. *The New England journal of medicine* **295**, 417-420.
4. Koenig RJ, Peterson CM, Kilo C *et al.* (1976) Hemoglobin AIc as an indicator of the degree of glucose intolerance in diabetes. *Diabetes* **25**, 230-232.
5. Koenig RJ, Blobstein SH, Cerami A (1977) Structure of carbohydrate of hemoglobin AIc. *The Journal of biological chemistry* **252**, 2992-2997.

6. Schmidt AM, Hori O, Brett J *et al.* (1994) Cellular receptors for advanced glycation end products. Implications for induction of oxidant stress and cellular dysfunction in the pathogenesis of vascular lesions. *Arteriosclerosis and thrombosis : a journal of vascular biology / American Heart Association* **14**, 1521-1528.
7. Sell DR, Monnier VM (1989) Isolation, purification and partial characterization of novel fluorophores from aging human insoluble collagen-rich tissue. *Connective tissue research* **19**, 77-92.
8. Monami M, Lamanna C, Lambertucci L *et al.* (2006) Fasting and post-prandial glycemia and their correlation with glycated hemoglobin in type 2 diabetes. *J Endocrinol Invest* **29**, 619-624.
9. Landgraf R (2004) The relationship of postprandial glucose to HbA1c. *Diabetes/Metabolism Research and Reviews* **20**, S9-S12.
10. Ahmed N, Babaei-Jadidi R, Howell SK *et al.* (2005) Glycated and Oxidized Protein Degradation Products Are Indicators of Fasting and Postprandial Hyperglycemia in Diabetes. *Diabetes Care* **28**, 2465-2471.
11. Beisswenger PJ, Howell SK, O'Dell RM *et al.* (2001) α-Dicarbonyls Increase in the Postprandial Period and Reflect the Degree of Hyperglycemia. *Diabetes Care* **24**, 726-732.
12. Brownlee M, Cerami A, Vlassara H (1988) Advanced Products of Nonenzymatic Glycosylation and the Pathogenesis of Diabetic Vascular-Disease. *Diabetes Metab Rev* **4**, 437-451.
13. Lepape A, Guitton JD, Muh JP (1981) Modifications of Glomerular Basement-Membrane Cross-Links in Experimental Diabetic Rats. *Biochem Bioph Res Co* **100**, 1214-1221.
14. Sensi M, Tanzi P, Bruno MR *et al.* (1986) Human Glomerular-Basement-Membrane - Altered Binding Characteristics Following Invitro Nonenzymatic Glycosylation. *Annals of the New York Academy of Sciences* **488**, 549-552.
15. Vlassara H (1996) Advanced glycation end-products and atherosclerosis. *Annals of medicine* **28**, 419-426.
16. Sell DR, Monnier VM (1989) Structure elucidation of a senescence cross-link from human extracellular matrix. Implication of pentoses in the aging process. *The Journal of biological chemistry* **264**, 21597-21602.
17. Monnier VM (1989) Toward a Maillard reaction theory of aging. *Progress in clinical and biological research* **304**, 1-22.
18. Bobbink IW, de Boer HC, Tekelenburg WL *et al.* (1997) Effect of extracellular matrix glycation on endothelial cell adhesion and spreading: involvement of vitronectin. *Diabetes* **46**, 87-93.
19. Kent MJ, Light ND, Bailey AJ (1985) Evidence for glucose-mediated covalent cross-linking of collagen after glycosylation in vitro. *The Biochemical journal* **225**, 745-752.
20. Tanaka S, Avigad G, Brodsky B *et al.* (1988) Glycation induces expansion of the molecular packing of collagen. *Journal of molecular biology* **203**, 495-505.
21. Sensi M, Tanzi P, Bruno MR *et al.* (1989) Nonenzymic glycation of isolated human glomerular basement membrane changes its physicochemical characteristics and binding properties. *Nephron* **52**, 222-226.
22. Klein RL, Laimins M, Lopes-Virella MF (1995) Isolation, characterization, and metabolism of the glycated and nonglycated subfractions of low-density lipoproteins isolated from type I diabetic patients and nondiabetic subjects. *Diabetes* **44**, 1093-1098.
23. Iwashima Y, Eto M, Hata A *et al.* (2000) Advanced glycation end products-induced gene expression of scavenger receptors in cultured human monocyte-derived macrophages. *Biochem Bioph Res Co* **277**, 368-380.
24. Basta G, Schmidt AM, De Caterina R (2004) Advanced glycation end products and vascular inflammation: implications for accelerated atherosclerosis in diabetes. *Cardiovascular research* **63**, 582-592.
25. Schmidt AM, Vianna M, Gerlach M *et al.* (1992) Isolation and characterization of two binding proteins for advanced glycosylation end products from bovine lung which are present on the endothelial cell surface. *The Journal of biological chemistry* **267**, 14987-14997.
26. Vlassara H, Li YM, Imani F *et al.* (1995) Identification of galectin-3 as a high-affinity binding protein for advanced glycation end products (AGE): a new member of the AGE-receptor complex. *Molecular medicine* **1**, 634-646.
27. Schmidt AM, Hori O, Chen JX *et al.* (1995) Advanced glycation endproducts interacting with their endothelial receptor induce expression of vascular cell adhesion molecule-1 (VCAM-1) in cultured human endothelial cells and in mice. A potential mechanism for the accelerated vasculopathy of diabetes. *The Journal of clinical investigation* **96**, 1395-1403.
28. Hofmann MA, Drury S, Fu CF *et al.* (1999) RAGE mediates a novel proinflammatory axis: A central cell surface receptor for S100/calgranulin polypeptides. *Cell* **97**, 889-901.

29. Kislinger T, Fu CF, Huber B *et al.* (1999) N-epsilon-(carboxymethyl)lysine adducts of proteins are ligands for receptor for advanced glycation end products that activate cell signaling pathways and modulate gene expression. *Journal of Biological Chemistry* **274**, 31740-31749.
30. Takeuchi M, Yamagishi S (2008) Possible involvement of advanced glycation end-products (AGEs) in the pathogenesis of Alzheimer's disease. *Current pharmaceutical design* **14**, 973-978.
31. Munch G, Westcott B, Menini T *et al.* (2012) Advanced glycation endproducts and their pathogenic roles in neurological disorders. *Amino Acids* **42**, 1221-1236.
32. Zhang QB, Ames JM, Smith RD *et al.* (2009) A Perspective on the Maillard Reaction and the Analysis of Protein Glycation by Mass Spectrometry: Probing the Pathogenesis of Chronic Disease. *J Proteome Res* **8**, 754-769.
33. Stitt AW (2001) Advanced glycation: an important pathological event in diabetic and age related ocular disease. *British Journal of Ophthalmology* **85**, 746-753.
34. Creager MA, Lüscher TF, of pwta *et al.* (2003) Diabetes and Vascular Disease: Pathophysiology, Clinical Consequences, and Medical Therapy: Part I. *Circulation* **108**, 1527-1532.
35. Mallidis C, Agbaje IM, Rogers DA *et al.* (2009) Advanced glycation end products accumulate in the reproductive tract of men with diabetes. *International journal of andrology* **32**, 295-305.
36. Diamanti-Kandarakis E, Piperi C, Patsouris E *et al.* (2007) Immunohistochemical localization of advanced glycation end-products (AGEs) and their receptor (RAGE) in polycystic and normal ovaries. *Histochemistry and cell biology* **127**, 581-589.
37. Mallidis C, Agbaje I, Rogers D *et al.* (2007) Distribution of the receptor for advanced glycation end products in the human male reproductive tract: prevalence in men with diabetes mellitus. *Human reproduction* **22**, 2169-2177.
38. Karimi J, Goodarzi MT, Tavilani H *et al.* (2012) Increased receptor for advanced glycation end products in spermatozoa of diabetic men and its association with sperm nuclear DNA fragmentation. *Andrologia* **44 Suppl 1**, 280-286.
39. Bonetti TC, Borges E, Jr., Braga DP *et al.* (2013) Intrafollicular soluble receptor for advanced glycation end products (sRAGE) and embryo quality in assisted reproduction. *Reproductive biomedicine online* **26**, 62-67.
40. Malickova K, Jarosova R, Rezabek K *et al.* (2010) Concentrations of sRAGE in serum and follicular fluid in assisted reproductive cycles--a preliminary study. *Clinical laboratory* **56**, 377-384.
41. Jinno M, Takeuchi M, Watanabe A *et al.* (2011) Advanced glycation end-products accumulation compromises embryonic development and achievement of pregnancy by assisted reproductive technology. *Human reproduction* **26**, 604-610.
42. Hao L, Noguchi S, Kamada Y *et al.* (2008) Adverse effects of advanced glycation end products on embryonal development. *Acta medica Okayama* **62**, 93-99.
43. Oliver EA, Buhimschi CS, Dulay AT *et al.* (2011) Activation of the receptor for advanced glycation end products system in women with severe preeclampsia. *The Journal of clinical endocrinology and metabolism* **96**, 689-698.
44. Naruse K, Sado T, Noguchi T *et al.* (2012) Peripheral RAGE (receptor for advanced glycation endproducts)-ligands in normal pregnancy and preeclampsia: novel markers of inflammatory response. *Journal of reproductive immunology* **93**, 69-74.
45. Cooke CL, Brockelsby JC, Baker PN *et al.* (2003) The receptor for advanced glycation end products (RAGE) is elevated in women with preeclampsia. *Hypertension in pregnancy : official journal of the International Society for the Study of Hypertension in Pregnancy* **22**, 173-184.
46. Fineschi S, De Cunto G, Facchinetti F *et al.* (2013) Receptor for advanced glycation end products contributes to postnatal pulmonary development and adult lung maintenance program in mice. *American journal of respiratory cell and molecular biology* **48**, 164-171.
47. Gul A, Rahman MA, Salim A *et al.* (2009) Advanced glycation end products in senile diabetic and nondiabetic patients with cataract. *Journal of diabetes and its complications* **23**, 343-348.
48. Gkogkolou P, Bohm M (2012) Advanced glycation end products: Key players in skin aging? *Dermato-endocrinology* **4**, 259-270.
49. Cai W, He JC, Zhu L *et al.* (2007) Reduced oxidant stress and extended lifespan in mice exposed to a low glycotoxin diet: association with increased AGER1 expression. *The American journal of pathology* **170**, 1893-1902.

50. Taylor A, Lipman RD, Jahngen-Hodge J *et al.* (1995) Dietary calorie restriction in the Emory mouse: effects on lifespan, eye lens cataract prevalence and progression, levels of ascorbate, glutathione, glucose, and glycohemoglobin, tail collagen breaktime, DNA and RNA oxidation, skin integrity, fecundity, and cancer. *Mechanisms of ageing and development* **79**, 33-57.
51. Reiser KM (1994) Influence of Age and Long-Term Dietary Restriction on Enzymatically Mediated Crosslinks and Nonenzymatic Glycation of Collagen in Mice. *Journal of Gerontology* **49**, B71-B79.
52. Mouton PR, Chachich ME, Quigley C *et al.* (2009) Caloric restriction attenuates amyloid deposition in middle-aged dtg APP/PS1 mice. *Neuroscience letters* **464**, 184-187.
53. Cai W, He JC, Zhu L *et al.* (2008) Oral glycotoxins determine the effects of calorie restriction on oxidant stress, age-related diseases, and lifespan. *The American journal of pathology* **173**, 327-336.
54. Stensen MH, Tanbo T, Storeng R *et al.* (2014) Advanced glycation end products and their receptor contribute to ovarian ageing. *Human reproduction* **29**, 125-134.
55. Smith MA, Sayre LM, Monnier VM *et al.* (1995) Radical AGEing in Alzheimer's disease. *Trends in neurosciences* **18**, 172-176.
56. Srikanth V, Maczurek A, Phan T *et al.* (2011) Advanced glycation endproducts and their receptor RAGE in Alzheimer's disease. *Neurobiology of aging* **32**, 763-777.
57. Vitek MP, Bhattacharya K, Glendening JM *et al.* (1994) Advanced glycation end products contribute to amyloidosis in Alzheimer disease. *Proceedings of the National Academy of Sciences of the United States of America* **91**, 4766-4770.
58. Bailey AJ, Sims TJ, Avery NC *et al.* (1993) Chemistry of collagen cross-links: glucose-mediated covalent cross-linking of type-IV collagen in lens capsules. *The Biochemical journal* **296 (Pt 2)**, 489-496.
59. Nicholl ID, Stitt AW, Moore JE *et al.* (1998) Increased levels of advanced glycation endproducts in the lenses and blood vessels of cigarette smokers. *Molecular medicine* **4**, 594-601.
60. American Diabetes A, European Association for the Study of D, International Federation of Clinical C *et al.* (2007) Consensus statement on the worldwide standardisation of the HbA1c measurement. *Diabetologia* **50**, 2042-2043.
61. American Diabetes A (2011) Diagnosis and classification of diabetes mellitus. *Diabetes Care* **34 Suppl 1**, S62-69.
62. Porta M, Sjoelie AK, Chaturvedi N *et al.* (2001) Risk factors for progression to proliferative diabetic retinopathy in the EURODIAB Prospective Complications Study. *Diabetologia* **44**, 2203-2209.
63. Klein R, Klein BE, Moss SE *et al.* (1994) The Wisconsin Epidemiologic Study of diabetic retinopathy. XIV. Ten-year incidence and progression of diabetic retinopathy. *Archives of ophthalmology* **112**, 1217-1228.
64. Klein R, Klein BE, Moss SE *et al.* (1998) The Wisconsin Epidemiologic Study of Diabetic Retinopathy: XVII. The 14-year incidence and progression of diabetic retinopathy and associated risk factors in type 1 diabetes. *Ophthalmology* **105**, 1801-1815.
65. Anonymous (1995) The relationship of glycemic exposure (HbA1c) to the risk of development and progression of retinopathy in the diabetes control and complications trial. *Diabetes* **44**, 968-983.
66. McCarter RJ, Hempe JM, Gomez R *et al.* (2004) Biological variation in HbA1c predicts risk of retinopathy and nephropathy in type 1 diabetes. *Diabetes Care* **27**, 1259-1264.
67. El-Salem K, Ammari F, Khader Y *et al.* (2007) Elevated glycosylated hemoglobin is associated with subclinical neuropathy in neurologically asymptomatic type-2 diabetic patients: A prospective study. *Eur J Neurol* **14**, 251-251.
68. Khaw KT, Wareham N, Bingham S *et al.* (2004) Association of hemoglobin A1c with cardiovascular disease and mortality in adults: the European prospective investigation into cancer in Norfolk. *Annals of internal medicine* **141**, 413-420.
69. Khaw KT, Wareham N, Bingham S *et al.* (2004) Preliminary communication: glycated hemoglobin, diabetes, and incident colorectal cancer in men and women: a prospective analysis from the European prospective investigation into cancer-Norfolk study. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology* **13**, 915-919.
70. Selvin E, Steffes MW, Zhu H *et al.* (2010) Glycated hemoglobin, diabetes, and cardiovascular risk in nondiabetic adults. *The New England journal of medicine* **362**, 800-811.
71. Selvin E, Coresh J, Shahar E *et al.* (2005) Glycaemia (haemoglobin A1c) and incident ischaemic stroke: the Atherosclerosis Risk in Communities (ARIC) Study. *Lancet neurology* **4**, 821-826.

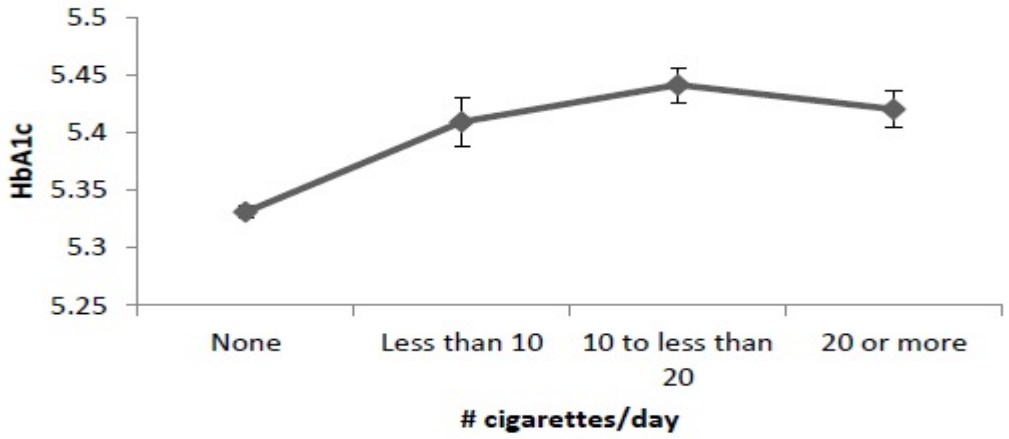
72. Matsushita K, Blecker S, Pazin A *et al.* (2010) The Association of Hemoglobin A1c With Incident Heart Failure Among People Without Diabetes: The Atherosclerosis Risk in Communities Study. *Diabetes* **59**, 2020-2026.
73. Selvin E, Rawlings AM, Grams M *et al.* (2014) Fructosamine and glycated albumin for risk stratification and prediction of incident diabetes and microvascular complications: a prospective cohort analysis of the Atherosclerosis Risk in Communities (ARIC) study. *The Lancet Diabetes & Endocrinology*.
74. Barr EL, Boyko EJ, Zimmet PZ *et al.* (2009) Continuous relationships between non-diabetic hyperglycaemia and both cardiovascular disease and all-cause mortality: the Australian Diabetes, Obesity, and Lifestyle (AusDiab) study. *Diabetologia* **52**, 415-424.
75. de Vegt F, Dekker JM, Ruhe HG *et al.* (1999) Hyperglycaemia is associated with all-cause and cardiovascular mortality in the Hoorn population: the Hoorn Study. *Diabetologia* **42**, 926-931.
76. van't Riet E, Rijkelijkhuizen JM, Alssema M *et al.* (2012) HbA1c is an independent predictor of non-fatal cardiovascular disease in a Caucasian population without diabetes: a 10-year follow-up of the Hoorn Study. *Eur J Prev Cardiol* **19**, 23-31.
77. Meigs JB, Nathan DM, D'Agostino RB *et al.* (2002) Fasting and postchallenge glycemia and cardiovascular disease risk - The framingham offspring study. *Diabetes Care* **25**, 1845-1850.
78. Park S, BarrettConnor E, Wingard DL *et al.* (1996) GHb is a better predictor at cardiovascular disease than fasting or postchallenge plasma glucose in women without diabetes - The Rancho Bernardo Study. *Diabetes Care* **19**, 450-456.
79. Pradhan AD, Rifai N, Buring JE *et al.* (2007) Hemoglobin a1c predicts diabetes but not cardiovascular disease in nondiabetic women. *Am J Med* **120**, 720-727.
80. Lin J, Ridker PM, Rifai N *et al.* (2006) A prospective study of hemoglobin A1c concentrations and risk of breast cancer in women. *Cancer Res* **66**, 2869-2875.
81. Blake GJ, Pradhan AD, Manson JE *et al.* (2004) Hemoglobin A(1c) level and future cardiovascular events among women. *Arch Intern Med* **164**, 757-761.
82. Sakurai M, Saitoh S, Miura K *et al.* (2013) HbA1c and the risks for all-cause and cardiovascular mortality in the general Japanese population: NIPPON DATA90. *Diabetes Care* **36**, 3759-3765.
83. Edwards BK, Ward E, Kohler BA *et al.* (2010) Annual report to the nation on the status of cancer, 1975-2006, featuring colorectal cancer trends and impact of interventions (risk factors, screening, and treatment) to reduce future rates. *Cancer* **116**, 544-573.
84. McPherson K, Steel CM, Dixon JM (2000) ABC of breast diseases. Breast cancer-epidemiology, risk factors, and genetics. *BMJ* **321**, 624-628.
85. Vlassopoulos A, Lean M, Combet E (2013) Influence of smoking and diet on glycated haemoglobin and 'pre-diabetes' categorisation: a cross-sectional analysis. *BMC Public Health* **13**, 1013.
86. Sargeant LA, Khaw K-T, Bingham S *et al.* (2001) Cigarette smoking and glycaemia: the EPIC-Norfolk Study. *International Journal of Epidemiology* **30**, 547-554.
87. Clair C, Bitton A, Meigs JB *et al.* (2011) Relationships of Cotinine and Self-Reported Cigarette Smoking With Hemoglobin A1c in the U.S.: Results from the National Health and Nutrition Examination Survey, 1999–2008. *Diabetes care* **34**, 2250-2255.
88. Guerin-Dubourg A, Catan A, Bourdon E *et al.* (2012) Structural modifications of human albumin in diabetes. *Diabetes & metabolism* **38**, 171-178.
89. Kawakami A, Kubota K, Yamada N *et al.* (2006) Identification and characterization of oxidized human serum albumin. *FEBS Journal* **273**, 3346-3357.
90. Rondeau P, Bourdon E (2011) The glycation of albumin: structural and functional impacts. *Biochimie* **93**, 645-658.
91. Varma SD, Devamanoharan PS (1991) Hydrogen peroxide in human blood. *Free Radic Res Commun* **14**, 125-131.
92. Lacy F, O'Connor DT, Schmid-Schönbein GW (1998) Plasma hydrogen peroxide production in hypertensives and normotensive subjects at genetic risk of hypertension. *Journal of hypertension* **16**, 291-303.
93. Frei B, Yamamoto Y, Niclas D *et al.* (1988) Evaluation of an isoluminol chemiluminescence assay for the detection of hydroperoxides in human blood plasma. *Analytical Biochemistry* **175**, 120-130.
94. Mueller S, Riedel H-D, Stremmel W (1997) Determination of Catalase Activity at Physiological Hydrogen Peroxide Concentrations. *Analytical Biochemistry* **245**, 55-60.

95. Stadtman ER, Levine RL (2000) Protein oxidation. *Ann N Y Acad Sci* **899**, 191-208.
96. Vlassopoulos A, Lean ME, Combet E (2013) Role of oxidative stress in physiological albumin glycation: a neglected interaction. *Free radical biology & medicine* **60**, 318-324.
97. Brownlee M, Vlassara H, Kooney A *et al.* (1986) Aminoguanidine prevents diabetes-induced arterial wall protein cross-linking. *Science* **232**, 1629-1632.
98. Ahmed N, Thornalley PJ (2002) Chromatographic assay of glycation adducts in human serum albumin glycated in vitro by derivatization with 6-aminoquinolyl-N-hydroxysuccinimidyl-carbamate and intrinsic fluorescence. *The Biochemical journal* **364**, 15-24.
99. Thornalley PJ (2003) Use of aminoguanidine (Pimagedine) to prevent the formation of advanced glycation endproducts. *Arch Biochem Biophys* **419**, 31-40.
100. Urios P, Grigorova-Borsos AM, Sternberg M (2007) Aspirin inhibits the formation of pentosidine, a cross-linking advanced glycation end product, in collagen. *Diabetes research and clinical practice* **77**, 337-340.
101. Menzel EJ, Reihsner R (1996) Comparison of the effect of different inhibitors of the non-enzymatic glycation of rat tail tendons and bovine serum albumin. *Annals of clinical biochemistry* **33 (Pt 3)**, 241-248.
102. Wu JW, Hsieh CL, Wang HY *et al.* (2009) Inhibitory effects of guava (Psidium guajava L.) leaf extracts and its active compounds on the glycation process of protein. *Food Chem* **113**, 78-84.
103. Choi SY, Jung SH, Lee HS *et al.* (2008) Glycation inhibitory activity and the identification of an active compound in Plantago asiatica extract. *Phytother Res* **22**, 323-329.
104. Kiho T, Usui S, Hirano K *et al.* (2004) Tomato paste fraction inhibiting the formation of advanced glycation end-products. *Bioscience, biotechnology, and biochemistry* **68**, 200-205.
105. Gugliucci A, Bastos DH, Schulze J *et al.* (2009) Caffeic and chlorogenic acids in Ilex paraguariensis extracts are the main inhibitors of AGE generation by methylglyoxal in model proteins. *Fitoterapia* **80**, 339-344.
106. Bixby M, Spieler L, Menini T *et al.* (2005) Ilex paraguariensis extracts are potent inhibitors of nitrosative stress: a comparative study with green tea and wines using a protein nitration model and mammalian cell cytotoxicity. *Life Sci* **77**, 345-358.
107. Ahmad MS, Pischetsrieder M, Ahmed N (2007) Aged garlic extract and S-allyl cysteine prevent formation of advanced glycation endproducts. *Eur J Pharmacol* **561**, 32-38.
108. Xi M, Hai C, Tang H *et al.* (2008) Antioxidant and antiglycation properties of total saponins extracted from traditional Chinese medicine used to treat diabetes mellitus. *Phytother Res* **22**, 228-237.
109. Stote KS, Baer DJ (2008) Tea consumption may improve biomarkers of insulin sensitivity and risk factors for diabetes. *J Nutr* **138**, 1584S-1588S.
110. Xie Y, Chen X (2013) Structures required of polyphenols for inhibiting advanced glycation end products formation. *Current drug metabolism* **14**, 414-431.
111. Crozier A, Jaganath IB, Clifford MN (2009) Dietary phenolics: chemistry, bioavailability and effects on health. *Natural product reports* **26**, 1001-1043.
112. Del Rio D, Costa LG, Lean ME *et al.* (2010) Polyphenols and health: what compounds are involved? *Nutr Metab Cardiovasc Dis* **20**, 1-6.
113. Rothwell JA, Urpi-Sarda M, Boto-Ordonez M *et al.* (2012) Phenol-Explorer 2.0: a major update of the Phenol-Explorer database integrating data on polyphenol metabolism and pharmacokinetics in humans and experimental animals. *Database : the journal of biological databases and curation* **2012**, bas031.
114. Bhagwat SA, Haytowitz, D.B., Holden, J.M. (2013) USDA database for the flavonoid content of selected foods, Release 3.1. USA: USDA.
115. Nettleton JA, Harnack LJ, Scrafford CG *et al.* (2006) Dietary flavonoids and flavonoid-rich foods are not associated with risk of type 2 diabetes in postmenopausal women. *J Nutr* **136**, 3039-3045.
116. Wedick NM, Pan A, Cassidy A *et al.* (2012) Dietary flavonoid intakes and risk of type 2 diabetes in US men and women. *Am J Clin Nutr* **95**, 925-933.
117. Song Y, Manson JE, Buring JE *et al.* (2005) Associations of dietary flavonoids with risk of type 2 diabetes, and markers of insulin resistance and systemic inflammation in women: a prospective study and cross-sectional analysis. *Journal of the American College of Nutrition* **24**, 376-384.
118. Basu A, Newman ED, Bryant AL *et al.* (2013) Pomegranate polyphenols lower lipid peroxidation in adults with type 2 diabetes but have no effects in healthy volunteers: a pilot study. *Journal of nutrition and metabolism* **2013**, 708381.

119. Celec P, Hodosy J, Palffy R *et al.* (2013) The short-term effects of soybean intake on oxidative and carbonyl stress in men and women. *Molecules* **18**, 5190-5200.
120. Cho Y-Y, Baek N-I, Chung H-G *et al.* (2012) Randomized controlled trial of Sajabalssuk (Artemisia princeps Pampanini) to treat pre-diabetes. *European Journal of Integrative Medicine* **4**, e299-e308.
121. Fukino Y, Ikeda A, Maruyama K *et al.* (2008) Randomized controlled trial for an effect of green tea-extract powder supplementation on glucose abnormalities. *European journal of clinical nutrition* **62**, 953-960.
122. Cicero AFG, Nascetti S, Lopez-Sabater MC *et al.* (2008) Changes in LDL fatty acid composition as a response to olive oil treatment are inversely related to lipid oxidative damage: The EUROLIVE study. *Journal of the American College of Nutrition* **27**, 314-320.
123. McNair ED, Wells CR, Qureshi M *et al.* (2010) Soluble Receptors for Advanced Glycation End Products (sRAGE) as a Predictor of Restenosis Following Percutaneous Coronary Intervention. *Clin Cardiol* **33**, 678-685.
124. Ng ZX, Chua KH, Iqbal T *et al.* (2013) Soluble Receptor for Advanced Glycation End-Product (sRAGE)/Pentosidine Ratio: A Potential Risk Factor Determinant for Type 2 Diabetic Retinopathy. *Int J Mol Sci* **14**, 7480-7491.

601

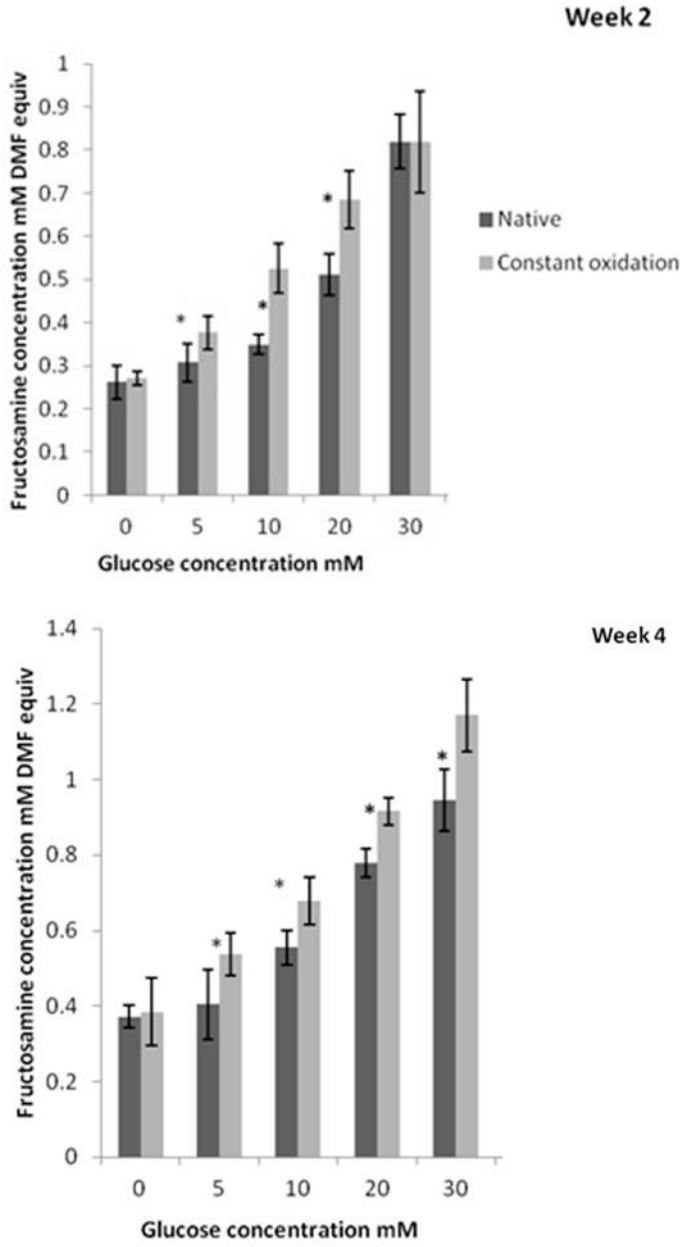
602 **Figure1.** Age-sex adjusted mean (SD) of %HbA1c according to number of cigarettes/day



603

604 **Figure as presented in Vlassopoulos et at 2013[85]**

Figure 2. Differences in fructosamine concentration after incubation with glucose alone compared to glucose and constant exposure to oxidation from hydrogen peroxide (10 nM) after two and four weeks incubation.



*p<0.05 native vs. constant oxidation ; fructosamine was measured using the nitroblue tetrazolium method with the synthetic fructosamine equivalent deoxy-morpholino-fructose (DMF) as a calibrator

Adapted from Vlassopoulos et al (2013)[96]