8. OXIDATIVE STRESS IN DIABETICS

Prof. Victor Blaton, Ph.D.
Department of Clinical Chemistry, Hospital AZ Sint-Jan AV, Brugge, Belgium

1.1. Introduction

Diabetes mellitus is a major source of morbidity in developed countries. Among its co-morbid conditions, atherosclerosis is perhaps the most important. Since the availability of insulin, up to three-quarters of all deaths among diabetics can be directly attributed to coronary artery disease (CAD). In patients with IDDM, up to one third will die of CAD by the age of 50 years. A number of known risk factors for CAD, such as hypertension, central obesity and dyslipidemia, are more common in diabetics than in the general population.

Thus diabetes represents a major contributing factor to the CAD burden in the developed world, and most of the excess attributed risk of CAD in diabetics cannot be readily quantified with the use of traditional risk factors analysis.

Diabetes is associated with a variety of metabolic abnormalities, principle among which is hyperglycaemia. The relation between hyperglycaemia and CAD is the subject of considerable debate because serum glucose does not consistently predict the existence of CAD. Presumably, this confusion sterns from the reliance on a simple blood glucose measurement, as recent prospective data have clearly established a link between a marker for chronic average glucose levels (HbA1c) and cardiovascular morbidity and mortality.

There is a considerable controversy with respect to the precise mechanism by which hyperglycaemia may contribute to the development of CAD in diabetes. There are established sequelae of hyperglycaemia, such as cytotoxicity, increased extracellular matrix production and vascular dysfunction and have all been implicated in the pathogenesis of diabetes–induced vascular disease.

Excess oxidative stress has captured considerable attention as a potential mechanism for the increased vascular disease in diabetics. The established association between atherosclerosis and lipid peroxidation within the vascular wall has led to a renewed interest in the oxidative stress of hyperglycaemia as a potential mechanism for diabetic vascular disease (Figure 1).

The molecular mechanism of biological oxidation by glucose was first identified in 1912 by Louis Maillard. This French chemist described a brown colour that formed from heating solutions of carbohydrates and amines and termed this process the “réaction du Maillard”. The reaction involves the combination of the aldehyde group of glucose with the amine group on proteins to form a Schiff-base followed by a rearrangement to form fructose-lysine.

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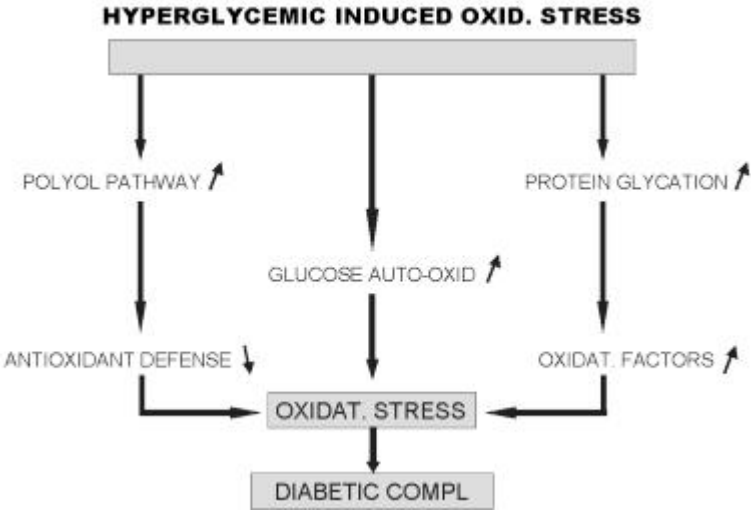
Figure 1. Hyperglycemic induced oxidative sress



This reversible glycosylation of aminogroups, or glycation, underlies the formation of HbA1c, the well-recognized marker of chronic glycaemic control in diabetes mellitus, but is not of any direct pathophysiological significance for the complications of diabetes. The final stage of the Maillard reaction involves the irreversible oxidation, or glucoxidation of fructoselysine to yield a host of advanced glycation end-products (AGEs) such as carboxymethyl-lysine, pentosidine and pyrroline, the formation of which correlate directly with the vascular and renal complications of diabetes mellitus. Unlike the quantitation of AGEs and AGE-modified proteins, the quantitation of lipid peroxidation in the setting of hyperglycaemia has been more problematic.

A novel class of prostanoid-like compounds, known collectively as F2-isoprostanes are specific non-enzymatic oxidation products of arachidonic acid and are subsequently released in the free form through the action of phospholipases.

In the quantification of oxidative stress, the determination of F2-isoprostanes has proved quite useful as a marker of lipid peroxidation both in vitro and in vivo. However the precise role of enhanced lipid peroxidation, and F2-isoprostanes in particular, in the vascular pathology associated with diabetes mellitus remains to be determined.

The simultaneous increased levels of 8-epi PGF2a in plasma and in urine of NIDDM and in IDDM as well tends to implicate hyperglycaemia as the culprit of metabolic derangement, since this is a major common feature of both patient populations. Improved glycaemic control reduces vascular oxidative stress, and has a profound influence on the degree of oxidative stress in diabetic patients.

In addition to AGE formation by oxidation of fructoselysine, there are other putative mechanisms that link hyperglycaemia to oxidative stress. The most direct is the autoxidation of glucose, which is subject to ene-diol rearrangement that results in the formation of an ene-diol radical ion. This species is capable to reduce molecular oxygen to form superoxide anion, which may contribute to the oxidation of lipids or to the activation of platelets. The dicarbonyl products are quite reactive and modify adjacent lysine groups to form AGEs such as n-(carboxymethyl)lysine. These reactions derived from glycose enolization are dependent on transition metal ions, and the availability of free, redox-active transition metal ions in vivo is controversial. Recent data demonstrating glycation-induced ceruloplasmin fragmentation and free copper release offer one possible mechanism for a source

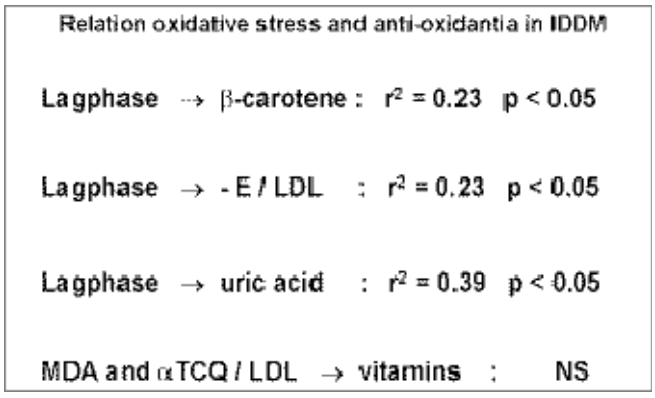
of extracellular transition metals. As an alternative mechanism of AGE-mediated oxidative stress, AGEs have also been shown to induce cellular lipid peroxidation through interacting with their specific surface receptor, and this effect can be attenuated by vitamin E.

Although there is a considerable evidence for increased lipid peroxidation in diabetes, arguments for a more generalized increase in oxidative stress are not secure. In vitro glycoxidation of collagen results in formation of AGEs as well as the protein oxidation products o-tyrosine and methionine sulphoxide. Diabetic patients demonstrate an increase in AGE formation compared with age-matched control subjects but no increase in the non-carbohydrate-derived protein oxidation products o-tyrosine and methionine sulphoxide. These data underscore the need for further investigation in to the precise molecular nature of oxidative stress in diabetes mellitus and the impact of such stress on diabetic vascular complications.

1.2. Experimental data in Type I diabetics

Patients with diabetes mellitus are particularly susceptible to morbidity and mortality resulting from cardiovascular diseases, especially atherosclerosis. Diabetes and coronary heart disease share many of the same risk factors, such as disorders of lipid metabolism and hypertension. The oxidation of low density lipoproteins (LDL) is considered a key event in the initiation of atherosclerosis. Although the exact mechanisms responsible for accelerated atherogenesis in patients with diabetes are not completely understood, an important role may be played by increased glycolisation of lipoproteins. Lipid abnormalities in diabetic patients are presented in Figure 2.

Figure 2. Lipid abnormalities in diabetic patients



The major aim of the study was to evaluate oxidative stress in well controlled type I diabetes without clinical complications. 36 patients, 19 males and 17 females were taken up in the protocol, aged 30 ± 9.7 years with 12.9 ± 6.8 years disease duration. Exclusion criteria for hypertension, vitamin supplementation or hypolipidaemic agents were applied. A control group of 37 persons, 15 males and 22 females, sex and age matched, without lipid abnormalities were compared. Besides basic biochemical analyses, more specific oxidative stress parameters were examined, as lag phase, TBARS and quanitation of ox-LDL with a monoclonal antibody (mAb-4EG) on ELISA. The major events were the differences in the serum lag phase between men and women (Figure 3), more pronounced in the diabetic patients and

were significantly correlated to differences in plasma copper and uric acid concentrations (Figure 4).

Figure 3. Differences in the serum lag phase between man and woman

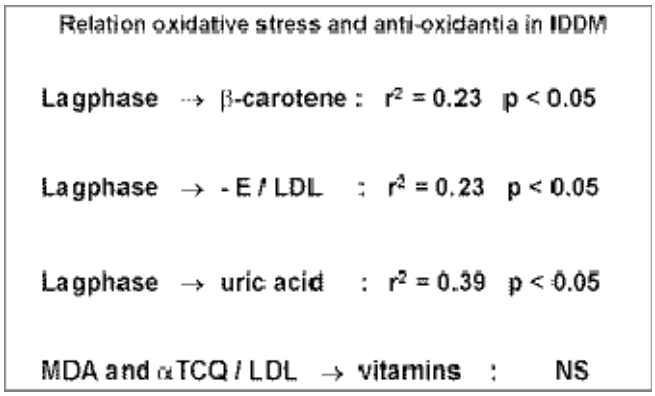


Figure 4. Relation oxidative stress and anti-oxidants in IDDM

Relation oxidative stress and anti-oxidantia in IDDM
Lagphase → β-carotene : r² = 0.23 p < 0.05
Lagphase → - E / LDL : r² = 0.23 p < 0.05
Lagphase → uric acid : r² = 0.39 p < 0.05
MDA and αTCQ / LDL → vitamins : NS

There was a direct relationship between the plasma lipid composition, ox-LDL and lag phase. A reciprocal value was obtained with the HDL value and the lag phase, probably due to changes in para-oxidase activity. There was no measured influence of serum vitamins on the oxidative stress parameters. The study conclusions were as follows:

1. There are significant differences in the lagphase between the control group and the well controlled IDDM patients.
2. There is a gender influence: females show a shorter lagphase against men, as well in the control group as in Type-I diabetics.
3. Between LDL-C, lagphase and the oxidation rate a significant correlation was observed. Similar findings were gained on LDL in vitro, although there was no positive realtionship with a-tocopherol.
4. HDL has a reciprocal value against the lagphase (r^2 = 0.38), explained by the high concentrations of lipidhydroperoxydes/HDL particle and the low con-centration of lipophilic antioxidants. HDLox > LDLox ?
5. The protective effect of HDL on LDLoxidation is determined by the paroxanase concentration, which is decreased in diabetic patient. LCAT and PAF-AH demonstrate similar protection.

6. OxLDL has a positive correlation with LDL-C and a negative one with HDL-C.
7. Lagphase and vitamin E are not correlated, there is a negative correlation between Vit. E/LDL and lagphase and no correlation between a-tocopherolquinone/LDL and oxLDL.

Recommended literature:

1. Astley S, Langrish-Smith A, Southon S, Sampson M. Vitamin E supplementation and oxidative damage to DNA and plasma LDL in type 1 diabetes. Diabetes Care 1999;22(10):1626-31.
2. Ruiz C, Alegria A, Barbera R, Farre R, Lagarda MJ. Lipid peroxidation and antioxidant enzyme activities in patients with type 1 diabetes mellitus. Scand J Clin Lab Invest 1999;59(2):99-105.
3. Ashour et al. Children type I - diabetes and chronic hyperglycaemia have a decreased antioxidant defense mechanism. J Clin Biochem Nutr 1999;26:99.
4. Maxwell et al. Predisposition of type I - diabetes to atherosclerosis is not determined by the anti-oxidative activity. Atherosclerosis 1997;89-96.
5. Tsai et al. Lagphase of LDL in type I - diabetes is lower than in controls. Diabetes 1994;1010-4.