*Workshop report***The role of anti-oxidative treatment in diabetes mellitus*****This meeting was held in Frankfurt am Main, Germany****Report prepared by L. Packer**

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In diabetic patients, the observed risk of complications in the vascular system exceeds that expected from the classic risk factors, which are known also as metabolic syndrome. Experimental and clinical findings suggest that enhanced levels of free radicals found in Type 1 (insulin-dependent) as well as in Type 2 (non-insulin-dependent) diabetic patients could be the risk factor explaining the excess mortality observed in these individuals. F. A. Gries (Diabetes-Research-Institute, Heinrich-Heine-University, Düsseldorf, Germany) stated. At a workshop held in Frankfurt am Main, a panel of international experts discussed the role of oxidative stress in the development of late diabetic complications and the implications for therapy.

These complications have long been assumed to be related to chronically elevated glucose levels. There are, however, doubts that hyperglycaemia contributes directly to the diabetic complications, as Dr. S. P. Wolff (University College and Middlesex School of Medicine, London, UK) pointed out, because the proposed pathogenetic mechanisms, e.g. glycation and aldose reductase activity, cannot explain all the clinical observations.

The reactions which occur when protein is exposed to glucose *in vitro* are considerably more complex than the simple addition of the sugar to protein amino groups. As Dr. Wolff explained, glucose, like other alpha-hydroxyaldehydes, can enolize. This enediol, catalysed by transition metals such as iron and copper, forms an endiol radical anion which under physiological conditions is oxidized by molecular oxygen to a ketoaldehyde. This glucose "autooxidation" yields free radicals, hydrogen peroxide and hydroxyl radicals. Evidence suggests that these compounds are a substantial cause of the structural damage because they mediate the reaction of various glucose reaction products (ketoaldehyde, Amadori adduct) with membrane proteins. Transition metals are believed to be of crucial importance in this cascade of reactions as they catalyse the autooxidation of glucose.

The damage which occurs when albumin is exposed to glucose under physiological conditions can be inhibited by catalase, free radical scavengers as well as metal chelating agents. The latter also inhibit protein browning induced by glucose. Interestingly, aldose reductase inhibitors also have metal chelating properties.

Common to all the metal-oxidizable low-molecular weight reductants such as glucose, vitamin C and polyunsaturated fatty acids is that in the presence of trace amounts of transition metals they undergo oxidation in the process of which a steady flux of free radicals is produced and thus contribute to enhanced oxidative stress.

Professor P. Roesen (Diabetes-Research-Institute, Heinrich-Heine-University, Düsseldorf, Germany) reported that there is good evidence that oxidative stress is increased in diabetic patients and that glucose can substantially contribute to the production of the reactive oxygen species (ROS) found in diabetic patients.

ROS, if not trapped by antioxidants such as vitamin E and C, glutathione or lipoic acid (which also has metal chelating properties) cause peroxidation of membrane lipids and induce oxidation of LDL which are cytotoxic for the endothelium thus, for example, increasing the permeability of the endothelium. ROS mediate the reaction of aldehydes (from lipid peroxide degradation and from glucose autooxidation) with membrane proteins to advanced glycation end-products which are also toxic for the endothelium (Fig. 1). Not only is the permeability of the endothelium by the action of ROS affected, the endothelial dependent vasorelaxation is also disturbed, characterised by a diminished nitric oxide (NO) and prostaglandin I release.

Furthermore, ROS can stimulate growth factors and induce the expression of adhesion molecules which are involved in the binding of platelets and monocytes to the endothelium. ROS also activate platelets with the release of platelet derived growth factor, thromboxane A₂ and 5-hydroxytryptophan the latter two being potent vasoconstrictors and capable of inducing vasospasms if the endothelium is injured. ROS can also activate monocytes which release cytokines such as interleukin 1 and tumour necrosis factor which are both cytotoxic to the endothelial cells. These blood cells also release superoxide anions and thus reinforce oxidative stress.

Fig. 1. Schematic pathogenetic concept of the development of vascular complications in diabetes and the protection by antioxidative treatment with tocopherol. Diabetes causes enhanced oxidative stress by several mechanisms (autooxidation of glucose, by formation of advanced glycation endproducts, activation of monocytes) which activates platelets and monocytes, causes endothelial dysfunction, and induces proliferation of smooth muscle cells (SMC). The type of vascular complication arising is strongly dependent on the anatomical site of endothelial damage: in large vessels → macroangiopathy; in capillaries → microangiopathy

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Fig. 2. Schematic diagram of the patho-biochemical situation in diabetes and the possible correction by oral administration of thioctic acid. Since glucose competitively inhibits the absorption of ascorbic acid into the cells. Thioctic acid (α -lipoic acid) increases the efficiency of the vitamin C-cycle and thereby also activates the vitamin E-cycle

Treatment with antioxidants such as vitamin E can significantly reduce these deleterious effects of ROS, Roesen emphasized. Antioxidants have also proven to be effective in preventing oxidative damage and protein cross-linking in lens crystallines as Professor E. Elstner (Institute of Botanic and Microbiology, Munich, Germany) was able to demonstrate.

A new study conducted by Dr. B. Kuklinski (Klinikum Suedstadt, Rostock, Germany) confirms the association of Longterm diabetic complications with increased peroxidation and enhanced oxidative stress. As Dr. Kuklinski reported, 80 normoglycaemic patients were randomly assigned to receive either alpha-lipoic acid (Thioctacid®; ASTA Medica AG, Frankfurt, Germany), D alpha-tocopherol, sodium selenite or no treatment. The patients who already suffered from at least two diabetic complications such as retinopathy, neuropathy and renal disease were treated for 12 weeks.

Antioxidant treatment reduced the peroxidation rate, characterized by malonic dialdehyde (MDA) levels which significantly differed from those in the control group. Associated with this decrease was a clear-cut improvement in vibratory sensibility of the distal extremities. The temperature sensibility demonstrated significantly higher rates of improvement during antioxidative treatment compared with the control group. Higher rates of improvements and lower rates of deterioration were also seen in the Achilles tendon and patellar reflexes in the treatment group. The distal symmetric polyneuropathy improved rapidly (patient groups did not include severe cases with night-time pain at rest) which emphasizes, according to Dr. Kuklinski, the need to initiate antioxidant supplementation at an early stage of the disease.

With treatment the progression of retinal microangiopathy appeared to be halted. There was no improvement seen in the control group and sodium selenite while the highest rate of change was achieved in the group treated with alpha lipoic acid. Antioxidant treatment also substantially decreased proteinuria and albuminuria.

Under normal conditions the organisms intrinsic defense mechanisms against free radicals (which can originate from endogenous sources such as phagocytosis as well as from exogenous sources such as radiation, smoking or alcohol) are sufficient to prevent harm. This may not be the case when synthesis of free radicals is increased. In diabetes, enhanced oxidative stress can be due to two mechanisms. On the one hand, free radicals are generated in the process of glucose autooxidation. On the other hand, the impaired regeneration of natural protective antioxidants such as vitamin E, vitamin C, glutathione and others is also a source of increased formation of free radicals. The regeneration of the reduced forms of these natural radical scavengers requires that NADPH be available in sufficient amounts. This may not be the case in diabetes since NADPH is consumed by the competing reduction of glucose to sorbitol in insulin-independent tissues under high glucose conditions.

Antioxidants in biological systems are in a very delicate balance, as Professor L. Packer (University of California, Berkeley, Calif.,

USA) pointed out. Amongst the naturally occurring antioxidants, ubiquinols, vitamin E and carotenoids are the major lipophilic agents that protect membranes and lipoproteins. In the aqueous phase the major water soluble antioxidants are vitamin C and glutathione. Alpha-lipoic acid is normally bound in enzymes such as alpha-ketodehydrogenases, but it can also act as an antioxidant with amphiphilic properties. Prof. Packer added that the interaction of these free radical scavengers is a prerequisite for restoring the body's antioxidant capacity especially in the presence of enhanced free radical formation. This, however, requires sufficiently high concentrations of these antioxidants.

When vitamin E interacts with a lipid radical, the vitamin E itself forms a radical, as Professor Packer showed in human LDL suspensions. To prevent the depletion of vitamin E which is located in the periphery of LDL, the vitamin E free radical can react with cellular reductants such as vitamin C (reduced ascorbic acid) which regenerates vitamin E.

A synergistic effect against the loss of vitamin E can be achieved by a combination of reduced lipoic acid (dihydrolipoic acid) and vitamin C. The observations indicate that dihydrolipoic acid can readily reduce oxidized vitamin C, Professor Packer concluded. This property of dihydrolipoic acid is due to its redox potential which is more negative than glutathione.

One, therefore, can expect that dihydrolipoic acid may even keep glutathione reduced but clearly reduces vitamin C, Professor Packer said.

There is another reason why patients with neuropathy may profit from treatment with lipoic acid. As Professor Packer explained, in diabetes glucose uptake into cells is impaired. This higher glucose also impairs uptake of dehydroascorbic acid (the oxidized form of vitamin C) which is taken up into the cell by the same sugar transport system that is used by glucose. However, if lipoic acid is administered, both lipoic acid and dihydrolipoic acid become elevated since this antioxidant uses the fatty acid transport system which is not affected by glucose elevation in diabetes. Thus, the efficiency of the vitamin C and vitamin E cycles can be increased (Fig. 2).

Oxidant radicals may also play a role in the initiation of Type 1 diabetes as Professor H. Kolb (Diabetes-Research-Institute, Heinrich-Heine University Düsseldorf, Germany) outlined. In vitro experiments, dihydrolipoic acid significantly inhibited macrophage-mediated islet cell lysis while in the absence of this antioxidant an increase in NO-mediated islet cell lysis could be demonstrated. The results indicate that lipoic acid can obviously protect islet cells effectively from oxygen radical attack.

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