Oxidative Stress and Diabetes

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ABSTRACT

Diabetes is a devastating disease throughout the world. It is associated with several mechanisms, one of which is oxidative stress. Oxidative stress plays an important role in the pathogenesis and the complications of diabetes. Hyperglycemia results in overproduction of oxygen free radicals, which contributes to the progression of diabetes. The development of complications during diabetes is also associated with oxidative stress. The cardiovascular complications, such as coronary artery diseases, peripheral vascular disease, and cerebrovascular disease, have been closely related to oxidative damage. The neurodegenerative disease as Alzheimer's disease has also been related to oxidative stress during diabetes. Consequently, antioxidant treatments have been proposed to be prospective in the treatment of diabetes. For example, glutathione reductase, glutathione peroxidase, glutathione, vitamins A, C, and E, catalase, and enzyme superoxide dismutase have been found t! o prevent the progression of diabetes and the occurrence of complications resulted from diabetes. In addition, physical exercise and insulin therapy can also improve diabetes through the reduction of oxidative stress.

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INTRODUCTION

Diabetes is a severe health problem that is increasing rapidly nowadays and is classified in two types, which are: type 1 diabetes also called juvenile onset diabetes, and type 2 diabetes, called non-insulin dependent diabetes. Diabetes is distinguished by a very high level of glucose in the body that causes deregulation of the metabolism. It has

been estimated that the number of people affected with diabetes in the world will increase to 300 million by 2025. The developed countries such as India, China, and the U.S. are presently the countries with the leading number of diabetics. Furthermore, seven percent of the residents of the United States are diabetics. Diabetes is the third leading fatal disorder after cancer and heart disease. With diabetes the body cannot regulate the amount of sugar in the blood.

Type 1 Diabetes

Patients with diabetes type 1 do not produce enough insulin or do not make it at all and cannot control the blood glucose level. Type 1 usually occurs in a person under 30 years of age. Insulin administration is required as well as the right amount of food. The symptoms of this disease are thirst, hunger and urination. Diabetes type 1 is accounting for 5%-10% of all cases of diabetes in the United States. Managing diabetes type 1 usually involves daily insulin treatment to sustain the patient's life (Standl et al., 2006). In Standl et al, research it was examined the incidence of nocturnal hypoglycemia and glycemic control following bedtime or morning insulin glargine plus glimepiride. In this 24-week, multinational, open, randomized study, 624 patients poorly controlled on oral therapy received morning or bedtime glargine plus morning glimepiride (2, 3 or 4 mg) titrated to a target fasting blood glucose level < or = 5.5 mmol/l. The incidence of nocturnal hypogly! cemia was equivalent between the two groups, with morning glargine non-inferior to bedtime. At endpoint, similar improvements in glycemic control were observed with morning compared to bedtime glargine. The endpoint mean daily glargine dose was comparable and there was no significant between-treatment difference in the

change in body weight. Once-daily glargine can be administered in a flexible morning or bedtime regimen to achieve good glycemic control without any difference in hypoglycemia (Standl et al., 2006).

Type 2 Diabetes

Type 2 diabetes is non-insulin dependent, and occurs to people that are 40 years of age and older and have a family history of diabetes. Type 2 diabetes phase is when the pancreas secretes insulin. However, the body is partially or absolutely incapable of using the insulin. Individuals with insulin resistance develop type 2 diabetes when they do not continue to produce enough insulin to cope with higher demands. This type of diabetes is treated through diet changes, exercise and a desirable glycemic control. All of the diet changes mentioned are needed because it helps manage the diabetes better and it also helps in experiencing a better feeling physically and mentally. Occasionally, oral medication or insulin is required in type 2.

Recently, oxidative stress has been associated with diabetes. Oxidative stress results from the overproduction of reactive oxygen species. Oxidative stress was found in diabetes as Sato et al, mentioned as far as 27 years ago. Many mechanisms that can result in excessive oxygen radical production lead to oxidative stress. In this review, we will analyze the role of oxidative stress in the development of diabetes.

OVERVIEW OF DIABETES (TYPE 1&2) AND OXIDATIVE STRESS

The human body is exposed to free radicals from outside the body (exogenous) and inside the body (endogenous). Some of the factors that lead to free radicals are smog, cigarette smoke, radiation, consumption of excessive amounts of alcohol, and even sunlight. Yet, some factors that led to free radicals come from within the body. The cells necessitate oxygen to produce the energy they need to work properly. In the process known as mitochondrial respiration, the cells take in oxygen, burn it, and release energy. During the process, free radicals are produced. Oxidative stress occurs when free radical production exceeds the body's ability to neutralize them. This imbalance happens for one of two reasons: a) when the antioxidant production is decreased, or, b) when the free radicals are produced in excess. For instance diabetes, or the aging process itself, can direct to increased speed of the production of these endogenous free radicals! and reduced antioxidant resistance. Oxidative stress functions on both sides, meaning that it help the progression and the development of diabetes and its complications (Ha and Lee, 2000). In the study of Ha et al, it was shown that oxidative stress is one of the important mediators of vascular complications in diabetes including nephropathy. High glucose produces reactive oxygen species as a result of glucose auto-oxidation, metabolism, and the development of advanced glycosylation end products. The concept of reactive oxygen species-induced tissue injury has currently been modified with the appreciation of new roles for reactive oxygen species in signaling pathways and gene expression. Although signal transduction pathways linking high glucose, reactive oxygen species, protein kinase C, transcription factors, and extracellular matrix protein synthesis in mesangial cells have not been fully clarified, the current data provide evidence that reactive oxygen species generate! d by glucose metabolism may act as integral signaling molecules under

high glucose as in other membrane receptor signaling (Ha and Lee, 2000).

Hyperglycemia, Diabetes and Oxidative Stress

Hyperglycemia is a connector between diabetes with diabetic complications (Brownlee, 2001; Rolo and Palmeira, 2006). In the review of Rolo et al, four of the most important molecular mechanisms have been involved in hyperglycemia-induced tissue damage: activation of protein kinase C isoforms through de novo synthesis of the lipid second messenger diacylglycerol increased hexosamine pathway flux, increased advanced glycation end product formation, and increased polyol pathway flux. Hyperglycemia-induced overproduction of superoxide is the causal link between high glucose and the pathways responsible for hyperglycemic damage. In fact, diabetes is typically associated with increased generation of free radicals and/or impaired antioxidant defense qualifications, representing a central contribution for reactive oxygen species in the onset, progression, and pathological consequences of diabetes. Besides oxidative stress, some evidence has demonstrated a link between various di! sturbances in mitochondrial functioning and type 2 diabetes. Mutations in mitochondrial DNA and decreases in mitochondrial DNA copy number have been connected to the pathogenesis of type 2 diabetes (Rolo and Palmeira, 2006). In addition, in the research of Brownlee et al, it was shown that hyperglycemia-induced process of overproduction of superoxide by the mitochondrial electron-transport chain by the four main molecular mechanisms and has been implicated in glucose-mediated vascular damage (Brownlee, 2001). Oxidative stress is increased in diabetes and is more definite in women and this leads to cardiovascular disease (Marra et al., 2002). In the study of Marra et al, it was examined whether type 1 diabetic patients with short duration of disease and without complications

have an altered oxidative status and whether there are differences between men and women. It was examined the oxidative status in 29 control subjects and 37 patients with no complications in diabetes typ! e 1. The duration of this process was between 3 to 9 years. Compared with the control subjects the individuals with type 1 diabetes had lower plasma antioxidant capacity, higher lipid hydroperoxide levels, higher total conjugated diene levels, lower 246 nm conjugated diene levels, and higher 232-nm conjugated diene levels. Compared with diabetic men, diabetic women had lower total plasma antioxidant capacity, higher lipid hydroperoxide levels, and lower 246 nm conjugated diene levels. These findings indicate that reduced antioxidant activity and increased oxidative stress occur early after the diagnosis of type 1 diabetes, especially in women, and this might explain the increased susceptibility of diabetic women to cardiovascular complications (Marra et al., 2002).

Modifications of life style through increased physical activity and reduced intake of calories can help lower the number of future cases of diabetes (Lakka et al., 2002). In Lakka et al, research, cardiovascular disease and all-cause mortality are at high risk in men with the metabolic syndrome, even in the absence of baseline cardiovascular disease and diabetes. Early recognition, treatment, and prevention of the metabolic syndrome present a major challenge for health care professionals confronting an epidemic of overweight and sedentary lifestyle (Lakka et al., 2002). People with diabetes do not have enough antioxidant defenses (Martin-Gallan et al., 2003), but, in contrast, too much of the free radical-induced damage. In Martin-Gallan et al, study, the purpose of the study was to ascertain the potential role of oxidative stress in the onset of disease-related pathophysiological complications in young type 1 diabetes patients. Indicative parameter!

s of lipoperoxidation, protein oxidation, and changes in antioxidant defense system status were measured in blood samples from 26 young diabetic patients with recently diagnosed (< 6 months) microangiopathy (+DC), 28 diabetic patients without complications (-DC), and 40 healthy age-matched controls (CR). Both diabetic groups presented similar fructosamine and glycated hemoglobin (HbA1c) values. Results showed erythrocyte glutathione peroxidase activity, glutathione content, and plasma beta-carotene to be significantly lower in diabetic patients compared with control subjects, but with no significant differences between -DC and +DC groups. Antioxidant enzyme superoxide dismutase activity was significantly higher in the erythrocytes of diabetic patients independently of the presence of microvascular complications. However, the plasma alpha-tocopherol/total lipids ratio was significantly diminished in +DC group compared with -DC (p =. 008). Lipid peroxidation indices measured ! in plasma-included malondialdehyde, lipid hydroperoxides, and lipoperoxides, which were significantly elevated in diabetic patients regardless of the presence of complications. Evidence of oxidative damage to proteins was shown both through the quantification of plasma protein carbonyl levels, which were significantly higher in -DC, and higher still in the +DC patients compared with those of controls and immunoblot analysis of protein-bound carbonyls. Additionally, a marked increase in protein oxidation was observed in +DC patients through assessment of advanced oxidation protein products (AOPP) considered to be an oxidized albumin index; AOPP values were significantly higher in +DC than in -DC patients (p <. 01) and CR (p <. 0001). These results point to oxidatively modified proteins as a differential factor possibly related to the pathogenesis of diabetic complications (Martin-Gallan et al., 2003).

Oxidative stress takes place while oxygen free radicals are produced in a very large amount through the diminution of oxygen. Diverse surveys were intended to establish the levels of stress associated biomarkers in type 1 (Varvarovska et al., 2003) and type 2 (Ceriello et al., 1998). In the survey made by Varvarovska et al. the results in diabetic children with type 1 diabetes showed extensively decreased glutathione peroxidase and plasma antioxidant capacity and increased malondialdehyde when compared with children that were healthy. Also almost the same findings were found in their siblings but not to the same degree. As a result, it is obvious that decreased antioxidative protection and simultaneous free radical overproduction takes place in diabetic children and that there is a not very important tendency in their siblings although it is similar. The conclusion is that is necessary reducing oxidative stress in diabetic children and postponing disease development in vuln! erable relatives (Varvarovska et al., 2003). In the survey of Ceriello et al, in type 2 diabetes, free radical production has been reported to be increased in diabetic patients and to be implicated in the development of diabetic complications. A meal was given to patients and after the meal, plasma malondialdehyde and vitamin C increased, while protein SH groups, uric acid, vitamin E, and total plasma radical-trapping parameter decreased more considerably in the diabetic subjects than in control subjects. This result demonstrates that in the absorptive phase, free radicals are produced in diabetic patients. Since plasma glucose arose extensively more in diabetic subjects than in control subjects, hyperglycemia may play a significant role in the generation of postprandial oxidative stress in diabetic patients (Ceriello et al., 1998).

Lipid Peroxidation and Diabetes

Indication of lipid peroxidation was experiential in diabetes type 2 through excessive plasma (Gopaul et al., 1995) and urine (Davi et al., 1999). The study of Gopaul et al, reports plasma levels of a specific nonenzymatic peroxidation product of arachidonic acid, esterified 8-epi-PGF2 alpha, from healthy- and non-insulin dependent diabetes mellitus individuals as an index of oxidative stress in vivo. Furthermore, it was studied some data which indicated that non-insulin dependent diabetes mellitus is connected with increased plasma lipid peroxidation (Gopaul et al., 1995). In the study of Davi et al, diabetes mellitus is associated with improved lipid peroxidation and lasting platelet activation. It was tested the hypothesis that the in vivo formation of the F2-isoprostane 8-iso-prostaglandin F2alpha, a bioactive product of arachidonic acid peroxidation, is improved in diabetes mellitus and contributes to platelet activation. Urine samples were ob! tained from 85 patients with the age of 85 and under and they were measured and tested in vivo index of platelet activation. It was concluded that diabetes mellitus is associated with increased formation of F2-isoprostanes, as an association of impaired glycemic control and enhanced lipid peroxidation. In addition, this may offer an important biochemical link between impaired glycemic control and persistent platelet activation. These results provide a rationale for dose-finding studies of antioxidant treatment in diabetes (Davi et al., 1999).

Possible Diabetes Complications

People with diabetes are more likely to get the diseases such as stroke

(Asfandiyarova et al., 2006), heart disease (Kamalesh, 2006) etc. In Asfandiyarova et al, study it was shown that stroke is a serious complication of diabetes but the risk factors for stroke in these patients are not fully defined. The aim of this retrospective study was to investigate the risk factors for stroke in patients with type 2 diabetes mellitus (T2DM). The group comprised 208 patients with T2DM, and the mean duration of follow-up was seven years (range 4-11 years). The incidence of stroke was investigated according to lymphocyte proliferation in response to insulin. Using cimetidine to inhibit cells with histamine receptors and indometacin to inhibit prostaglandin-synthesizing cells, a higher incidence of stroke was found in patients with indirect cell-mediated immunity to insulin. Therefore, one of the risk factors for stroke in patients with type 2 diabetes mellitus is! high activity of cells with histamine receptors and prostaglandin-synthesizing cells. These cells suppress cell-mediated immunity to insulin and may have a role in promoting the development of insulin resistance (Asfandiyarova et al., 2006). In a review written by Kamalesh et al, about heart disease it has been shown that the incidence of diabetes among patients with congestive heart failure (CHF) is increasing. Despite advances in therapy for CHF, mortality remains about 30% higher for diabetics with CHF than non-diabetics. Multiple mechanisms are responsible for development of CHF in diabetes with ischemic heart disease and its attendant complication of left ventricular dysfunction playing a major role. In the foreseeable future, it appears that physicians will have to deal with increasing numbers of subjects with diabetes, coronary disease and heart failure. Management of diabetes and co-morbid conditions plays a vital role in the prevention of development CHF in subjec! ts with diabetes. In addition, treatment of asymptomatic left ventricular dysfunction and management of CHF has been evolving over last few years

with major clinical trials (Kamalesh, 2006).

Damages Induced by Oxidative Stress

Oxidative stress makes damages in tissue (Hsieh et al., 2005), or organ (Molnar et al., 2004), caused by free radicals. Hsieh et al, has shown in his study that the possibility of 8-hydroxy-2'-deoxyguanosine (8-OHdG) serving as a sensitive biomarker of oxidative DNA damage and oxidative stress. Reactive oxygen species (ROS) have been reported to be a cause of diabetes induced by chemicals such as streptozotocin (STZ) in experimental animals. In the study, it was examined oxidative DNA damage in multiple tissues in rats with STZ-induced diabetes by measuring the levels of 8-OHdG in the liver, kidney, pancreas, brain, and heart. Levels of 8-OHdG in mitochondrial DNA (mtDNA) and nuclear DNA (nDNA) were also determined in multiple tissues of rats treated with rice bran oil. Levels of mtDNA of 8-OHdG were 10 times higher than those of nDNA in multiple tissues. Significant reductions in mtDNA 8-OHdG levels were seen in the liver, kidney, and pancreas ! of diabetic rats treated with rice bran oil compared with diabetic rats without intervention. The study demonstrated that oxidative mtDNA damage might occur in multiple tissues of STZ-induced diabetics rats and that intervention with rice bran oil treatment may reverse the increase in the frequency of 8-OHdG (Hsieh et al., 2005). In the study made by Molnar et al, angiotensin-converting enzyme (ACE) gene insertion/deletion (I/D) polymorphism is a well-known risk factor of hypertension, cardiovascular diseases and progression of diabetic nephropathy. In carriers of allele D, serum level of angiotensin-II is higher, which can be associated with

increased oxidative stress and subsequent endothelial damage. Albuminuria is a sensitive marker of endothelial damage, while serum activity of the enzyme gamma-glutamyl transferase--that plays important role in the antioxidant defense--may refer to the level of oxidative stress. This research reports on a cross-sectional clinical study! , where authors have examined on the relation between ACE gene insertion/deletion polymorphism and carbohydrate metabolism, hypertension as well as albuminuria in type 2 diabetics (n = 145). In patients carrying allele D, fructosamine levels were significantly higher (p = 0.007) than in carriers of allele I. Patients with II + ID genotypes and those who were treated with insulin took more antihypertensive drugs than the ones with II genotype or orally treated (p = 0.015). They found a significant association between genotype and fructosamine level (p = 0.023). Association between genotype or modality of treatment of diabetes (oral vs. insulin) and combined treatment of hypertension was of borderline significance. They found that fructosamin level of patients receiving ACE inhibitor was lower than that of patients not receiving ACE inhibitors. In patients with allele D, they have also found higher activity of gamma-GT and higher albuminuria. From this results and data of the literature the authors conclude that because of insulin resistance (in! connection with the presence of allele D), these patients tend to have a worse metabolic state, more advanced glycation products, due to which oxidative stress and endothelial cell damage may develop. As albuminuria and activity of gamma-GT were both found higher in patients with allele D, and the patients did not suffer of any hepatic disease, authors take the consequence that gamma-GT is a marker of the oxidative stress caused by allele D. Endothelial damage may explain that these patients take a higher number of antihypertensive combination. Based on this, D allele may contribute--via increased

glycation and oxidative stress--to the target organ damage in type 2 diabetes (Molnar et al., 2004).

OXIDATIVE STRESS AND DIABETIC COMPLICATIONS

In Ceriello et al, study, Ceriello examines facts that involve hyperglycemia-derived oxygen free radicals as mediators of diabetes-associated complications. Current studies have specified that a hyperglycemia-induced overproduction of superoxide appears to be the major event in the development of complications of diabetes. Superoxide overproduction is associated with increased generation of nitric oxide and, as a result, formation of the strong oxidant peroxynitrite and by poly (adenosine diphosphate-ribose) polymerase activation, which in turn further initiates the pathways implicated in the development of diabetes-related complications. In addition, this procedure consequence in severe endothelial dysfunction and initiation of inflammation in blood vessels of individuals with diabetes, and these aspects contribute to the development of complications of diabetes. Furthermore, in vivo evidence supports the major contribution of hyperglycemia in ! producing oxidative stress and, eventually, severe endothelial dysfunction in blood vessels of individuals with diabetes (Ceriello, 2006). In diabetes mellitus, persistent hyperglycemia forms several biochemical sequela, and diabetes induced oxidative stress may possibly play an important role in the beginning and progression of the disease.

Insulin Treatment and Diabetes Complications

The overproduction of reactive oxygen species leads to oxidative stress, in which reactive oxygen species consist of oxygen free radicals and free radicals cause oxidative stress. Insulin treatment stops successfully the onset and reduces the development of lasting diabetic complications in insulin-dependent diabetes mellitus, but the clinical management which is accessible for keeping tight control of glucose homeostasis does not decreases their occurrence (Group, 1993). In the Diabetes Control and Complications Trial Research Group et al, it gas been examined whether intensive treatment with the goal of maintaining blood glucose concentrations close to the normal range could decrease the frequency and severity of complications. A total of 1441 patients with insulin-dependent diabetes mellitus (IDDM)--726 with no retinopathy at base line and 715 with mild retinopathy were randomly assigned to intensive therapy administered either with an external insulin pump or by thr! ee or more daily insulin injections and guided by frequent blood glucose monitoring or to conventional therapy with one or two daily insulin injections. The patients were followed for a mean of 6.5 years, and the appearance and progression of retinopathy and other complications were assessed regularly. In the primary-prevention cohort, intensive therapy reduced the adjusted mean risk for the development of retinopathy by 76 percent, as compared with conventional therapy. In the secondary-intervention cohort, intensive therapy slowed the progression of retinopathy by 54 percent and reduced the development of proliferative or severe nonproliferative retinopathy by 47 percent. In the two cohorts combined, intensive therapy reduced the occurrence of microalbuminuria by 39, that of albuminuria by 54

percent, and that of clinical neuropathy by 60 percent. The chief adverse event associated with intensive therapy was a two-to-threefold increase in severe hypoglycemia. Intensive th! erapy effectively delays the onset and slows the progression of diabetic retinopathy, nephropathy, and neuropathy in patients with IDDM (Group, 1993).

Physiologic and Pathophysiologic Procedures

A variety of physiologic and pathophysiologic procedures are believed that reactive oxygen species play an important part in which the expansion of oxidative stress may have a significant function in disease mechanisms. A common pathogenic method in quite a few complications of diabetes such as nephropathy, retinopathy, and atherosclerosis, is too much oxidative stress, which happens as a consequence of an imbalance at the cellular level involving production and abolition of reactive oxygen species (Zhang et al., 2003; Voziyan and Hudson, 2005). In the study of Zhang et al, it was shown that vascular NAD (P) H oxidase-derived reactive oxygen species (ROS) such as hydrogen peroxide (H2O2) have emerged as important molecules in the pathogenesis of atherosclerosis, hypertension, and diabetic vascular complications. In addition, myeloperoxidase (MPO), a transcytosable heme protein that is derived from leukocytes, is also believed to play important roles in the above-mentioned i! nflammatory vascular diseases. Previous studies have shown that MPO-induced vascular injury responses are H2O2 dependent. It is well known that MPO can use leukocyte-derived H2O2; however, it is unknown whether the vascular-bound MPO can use vascular nonleukocyte oxidase-derived H2O2 to induce vascular injury. In the present study, ANG II was used to

stimulate vascular NAD (P) H oxidase and increase their H2O2 production in the vascular wall, and vascular dysfunction was used as the vascular injury parameter. It was demonstrated that vascular-bound MPO has sustained activity in the vasculature. MPO could use the vascular NAD (P) H oxidase-derived H2O2 to produce hypochlorus acid (HOCl) and its chlorinating species. More importantly, MPO derived HOCl and chlorinating species amplified the H2O2-induced vascular injury by additional impairment of endothelium-dependent relaxation. HOCl-modified low-density lipoprotein protein (LDL), a specific biomarker for the MPO-HOCl-chlorin! ating species pathway, was expressed in LDL and MPO-bound vessels with vascular NAD (P)H oxidase-derived H2O2. MPO-vascular NAD(P)H oxidase-HOCl-chlorinating species may represent a common pathogenic pathway in vascular diseases and a new mechanism involved in exacerbation of vascular diseases under inflammatory conditions (Zhang et al., 2003).

Function of Oxidative Stress

The function of oxidative stress in the progression of clinical complications in young patients with diabetes has been barely reported. In some research it was found that expanded levels of oxidative stress in children and adolescents that were diagnosed with diabetes with no complications proposed a high level of oxidant stress (Dominguez et al., 1998). In the research made by Dominguez et al, the persistence of hyperglycemia has been reported to cause increased production of oxygen free radicals through glucose auto oxidation and nonenzymatic glycation. Furthermore, the purpose of this study was to

establish whether oxidative cellular damage takes place at the clinical onset of diabetes and in later stages of the disease in young patients such as children and adolescents. Indicative parameters of lipoperoxidation, protein oxidation, and modifications in the status of antioxidant defense systems were estimated in single blood samples from 54 diabetic children, adolescents, ! and young adults and 60 healthy age- and sex-matched control subjects. Malondialdehyde and protein carbonyl group levels in plasma were increasingly higher in diabetic children and adolescents than in control subjects. In diabetic children at onset of clinical diabetes was found the highest erythrocyte superoxide dismutase activity and in adolescents, superoxide dismutase activity was also higher comparative to the control subjects. In contrast, compared with control subjects, erythrocyte glutathione peroxidase was extensively lower in diabetic children and adolescents. At the current onset of diabetes it was found a considerable decline in blood glutathione content. In addition, the results demonstrated progressive depletion during diabetes development. This study had shown that in diabetic patients that were examined the systematic oxidative stress was present on the early onset of type 1 diabetes and is higher in early adulthood. Decreased antioxidant defenses may increa! se the susceptibility of diabetic patients to oxidative injury. Appropriate support for enhancing antioxidant supply in these young diabetic patients may help prevent clinical complications during the course of the disease (Dominguez et al., 1998).

Hyperglycemia and Oxidative Stress

Hyperglycemia leads to some important complications through oxidative stress in

many cells. Free radicals make an important contribution in the development of diabetes complications (Hsu et al., 2006) such as changes in kidney, nerve, vascular tissue etc. In the research made by Hsu et al, parameters of lipid peroxidation, protein oxidation, and antioxidant defense systems were measured in blood samples from 47 children with type 1 diabetes mellitus and from 51 healthy controls, matched for age and sex. In the children with diabetes chemiluminescent assay of plasma superoxide anion gave photoemission, which were considerably higher than those in controls. In addition, plasma vitamin A levels in children with diabetes were also higher than those in controls. In a subgroup of 24 diabetic children with blood HbA1C levels >or=8.5%, plasma lipoperoxide and vitamin E levels were higher ($p < 0.05$) than those in 23 diabetic children with blood HbA1C levels <8.5%. In a subgroup of 26! children with diabetes duration >or=5 yr, plasma lipoperoxide levels were higher ($p < 0.05$) than those in 21 children with diabetes duration <5 yr. These results confirm the presence of oxidant stress in children with type 1 diabetes mellitus and prove that particular manifestations of oxidant stress are influenced by the duration of diabetes and by the efficiency of glycemic control. These remarks propose that supportive therapy intended at oxidative stress may help to prevent clinical complications in children with type 1 diabetes mellitus (Hsu et al., 2006).

Diabetic Complications

Some of the diabetic complications that are going to be discussed are as follows: cardiovascular complications and (Haidara et al., 2006) and neuropathy complications (Martin et al., 2006) which includes the Alzheimer's disease. In the review written by

Haidara et al, it has been found that diabetes is an important risk factor for the development of cardiovascular problems such as coronary heart disease, peripheral arterial disease, hypertension, stroke, cardiomyopathy, nephropathy and retinopathy. Furthermore, a linking element between all this complications could be the excess production of reactive oxygen species (Haidara et al., 2006). In the study of Martin et al, the objective was to evaluate the impact of prior intensive diabetes therapy on neuropathy among former Diabetes Control and Complications Trial (DCCT) participants. At the conclusion of the DCCT, subjects in the intensive group were encouraged to maintain intensive therapy, and subjects in the conventi! onal group were encouraged to begin intensive therapy. Thereafter, we annually assessed neuropathy as part of the Epidemiology of Diabetes Intervention and Complications (EDIC) study. Neuropathy was defined using the Michigan Neuropathy Screening Instrument (MNSI). It was recorded potential adverse consequences of neuropathy. At the first EDIC examination, 1,257 subjects participated in the neuropathy assessment. Consistent with DCCT results, the former intensive group showed a lower prevalence of neuropathy than the conventional group based on positive questionnaire (1.8 vs. 4.7%; $P = 0.003$) or examination (17.8 vs. 28.0%; $P < 0.0001$) results. Despite similar levels of glycemic control, symptoms and signs of neuropathy remained less prevalent among the former intensive group compared with the conventional group. At the beginning of the EDIC study, prior intensive therapy reduced the odds of having symptoms and signs of neuropathy using MNSI criteria by 64% ($P = 0.0044$) and! 45% ($P < 0.0001$), respectively, with similar odds reductions observed for both neuropathic symptoms (51%, $P < 0.0001$) and neuropathic signs (43%, $P < 0.0001$) across 8 years of EDIC follow-up.

The benefits of 6.5 years of intensive therapy on neuropathy status extended for at least 8 years beyond the end of the DCCT, similar to the findings described for diabetic retinopathy and nephropathy (Martin et al., 2006).

OXIDATIVE STRESS AND DIABETIC COMPLICATIONS

Cardiovascular complications are major causes of death and diabetes disease. Oxidative stress is a regular characteristic of diabetic complications when the action of antioxidant systems is overwhelmed by additional production of reactive oxygen species (Jones, 2006). In the review of Jones et al, it is stated that oxidative stress in aging can result from an imbalance of prooxidants and antioxidants with excessive, destructive free radical chemistry. In addition, several studies in collaboration with the Emory Clinical Biomarkers Laboratory show that the redox state of the central tissue antioxidant, glutathione (GSH), can be measured in human plasma and provides a quantitative systemic indicator of oxidative stress. Furthermore, in vitro studies show that variation in cysteine/cysteine redox over the range found in vivo affects signaling pathways, which control cell proliferation and oxidant-induced apoptosis. As a conclusion free radical scavengi! ng antioxidants are of increased importance when thiol/disulfide redox states are oxidized (Jones, 2006). Due to the expansion of macro vascular and micro vascular complications, the morbidity and mortality of diabetes is increasing (Basu et al., 2005). In the study of Basu et al, macro-and micro-vascular complications are important causes of mortality and morbidity. Moreover, micro-albuminuria is a surrogate marker for detection of vasculopathy for which early detection and aggressive treatment can reduce mortality

and morbidity. A small study was conducted to identify the usefulness of this marker, which can be used as a cost-effective tool for detecting the dreadful complication early. This study has demonstrated that in presence of micro-albuminuria the vascular complications of diabetes definitely increase and it is more relevant in cases of type 2 diabetes irrespectively of other parameters rendering it to be an independent risk factor. It also indicates that presence! of this marker along with vasculopathy is time dependent ie, more the duration of the disease more is the complication (Basu et al., 2005).

The factors that increase the possibility of cardiovascular disease and that come from insulin resistance are obesity (Bacha et al., 2006), dyslipidemia, and hypertension (Shaw et al., 2006). In the study of Bacha et al, obesity is often associated with insulin resistance and the components of the metabolic syndrome. However, wide variations in insulin sensitivity are noted in obese youth. It is not clear if greater insulin resistance confers a higher risk of cardiovascular comorbidities and risk for type 2 diabetes. It has been investigated physical and metabolic features of 54 obese adolescents. Subsequently, it was pair matched 17 moderately insulin-resistant (MIR group) to 17 severely insulin-resistant (SIR group) youth based on cut points for insulin sensitivity. It has been evaluated differences in body composition, abdominal fat, cardiorespiratory fitness (CRF), insulin sensitivity and secretion, substrate utilization, and fasting adiponectin and lipid profile. SIR yo! uth had higher visceral adiposity (78.3 +/- 6.9 vs. 60.3 +/- 6.9 cm(2), P = 0.017) and waist-to-hip ratio (0.91 +/- 0.01 vs. 0.86 +/- 0.02, P = 0.026) and lower HDL (1.0 +/- 0.03 vs. 1.16 +/- 0.06 mmol/l, P = 0.015) than pair-matched MIR subjects. There was a tendency for adiponectin and CRF to be lower in SIR subjects. SIR youth also had an impaired balance between insulin sensitivity and beta-cell

compensation with a lower glucose disposition index. Despite similar body mass index, the degree of insulin resistance impacts the risk for obesity-related metabolic comorbidities. The SIR youth are at greater risk for type 2 diabetes and cardiovascular disease (Bacha et al., 2006). In the study of Shaw et al, the metabolic syndrome represents a constellation of risk factors caused by insulin resistance, dyslipidemia, hypertension, and obesity, resulting in elevated coronary disease risk. From a multicenter prospective registry of 7,849 patients, the relation among the metabolic! syndrome, diabetes, and risk stratification with stress technetium-99m tetrofosmin single photon-emission computed tomography (SPECT) was evaluated. The percentage of stress myocardial defects was calculated as < or = 5%, 5.1% to 10%, 10.1% to 15%, and > 15%. A Cox proportional-hazards model was used to estimate cardiovascular death or myocardial infarction (n = 752). Of 7,849 patients, 42% had the metabolic syndrome. Patients with the metabolic syndrome had an 84% 2-year event-free survival rate, lower than patients with normal metabolic status (p <0.0001). In patients with the metabolic syndrome, the percentage of moderate to severely abnormal SPECT findings ranged from 11% to 44% for those with 3 to 5 risk factors for the metabolic syndrome. There was an additive relation between the number of risk factors for the metabolic syndrome and the extent and severity of abnormalities in SPECT findings (p <0.0001). Patients with 5 risk factors for the metabolic syndrome were at the greatest risk, with hazard ratios from 7.8- to 14.1-fold for mild t! o severely abnormal SPECT findings. For diabetic patients requiring combined oral and insulin therapy, relative risk ratios increased from 15 to 21.4 for patients with > 5% to > 15% stress myocardial perfusion defects. In conclusion, cardiovascular prognosis is affected by the degree of metabolic dysfunction, and stress-

induced reductions in myocardial perfusion provide an accurate means for near-term risk stratification (Shaw et al., 2006).

Diabetes is a genetic makeup of the cardiovascular disease (Laakso, 1999). In Laasko et al, review it is specified that cardiovascular disease is the most important cause of mortality and morbidity among patients with type 2 diabetes. In addition, conventional risk factors take part similarly to macrovascular complications in patients with type 2 diabetes and nondiabetic subjects, and therefore, other explanations have been required for enhanced atherothrombosis in type 2 diabetes (Laakso, 1999). In Yamagishi et al, review cardiovascular disease is the cause of disabilities and death in diabetes. In addition, diabetes is linked with a high increase in the risk of atherosclerotic vascular disorders, including coronary, peripheral artery and cerebrovascular disease (Yamagishi et al., 2006).

Coronary Artery Disease in Diabetes

Diabetes mellitus corresponds to a main coronary risk factor (Norhammar et al., 2004). In the study of Norhmmar et al, diabetes mellitus is a major contributor to coronary artery disease. In spite of the progress in the management of patients with unstable coronary syndromes, this situation is still connected to a significantly increased mortality and morbidity among diabetic patients. Current data advocates early revascularization in unstable coronary syndromes. Moreover, diabetic patients subjected to coronary interventions under stable conditions have a higher risk for complications and a more dismal prognosis than nondiabetic subjects. Accordingly, it

is of significant interest to obtain further information regarding the best possible management of diabetic patients with unstable coronary artery disease. The conclusion of this research was that an invasive strategy improved outcome for both diabetic and nondiabetic patients with unstable c! oronary artery disease. However, diabetes mellitus still is an independent and significant risk factor for death and myocardial infarction in the invasive group. Thus, factors beyond the extent of flow-limiting coronary lesions are of considerable importance for outcome in diabetic subjects with unstable coronary syndromes (Norhammar et al., 2004). Diabetes type 2 helps atherosclerotic plaque progress throughout diverse methods between which oxidative stress seems to play a main part stimulated by hyperglycemia (Basta et al., 2004). In the review of Basta et al, the formation of advanced glycation end products (AGEs) is an important biochemical abnormality that accompanies diabetes mellitus and, likely, inflammation in general. Furthermore, driven by hyperglycemia and oxidant stress, AGEs form to a greatly accelerated degree in diabetes. Within the vessel wall, collagen-linked AGEs may "trap" plasma proteins, quench nitric oxide (NO) activity and interact with specific rece! ptors to modulate a large number of cellular properties. On plasma low-density lipoproteins (LDL), AGEs initiate oxidative reactions that promote the formation of oxidized LDL. Interaction of AGEs with endothelial cells as well as with other cells accumulating within the atherosclerotic plaque, such as mononuclear phagocytes and smooth muscle cells (SMCs) provides a mechanism to augment vascular dysfunction. Specifically, the interaction of AGEs with vessel wall components increases vascular permeability, the expression of procoagulant activity and the generation of reactive oxygen species

(ROS), resulting in increased endothelial expression of endothelial leukocyte adhesion molecules. AGEs potently modulate initiating steps in atherogenesis involving blood-vessel wall interactions, triggering an inflammatory-proliferative process and, furthermore, critically contribute to propagation of inflammation and vascular perturbation in established disease. Thus, a better understanding of the biochemical mechanisms by which AGEs contribute to such pro! cesses in the vessel wall could be relevant to devise preventive and therapeutic strategies for diabetic atherosclerosis (Basta et al., 2004). A constricted management of cardiovascular risk factors is supposed to recommend prescriptions of drugs with the purpose of stabilization or even reduction of atherosclerosis. In addition, these drugs consist of statines, angiotensin converting enzyme inhibitors and aspirin combined with clopidogrel (Ludwig and Shen, 2006). In Ludwig et al, study, Landmark clinical trials indicated that statins effectively reduced cardiac death and events in patients with coronary artery disease or diabetes mellitus (DM). The benefits of statins on the prevention of vascular events were independent from age, sex or baseline lipid levels in diabetic patients. Statins not only prevent atherosclerotic macrovascular complications, but also postpone the development of microvascular complications of DM, such as nephropathy and retinopathy. The non-choleste! rol lowering or pleiotropic effects of statins have attracted vast attention. Results from experimental and clinical studies suggest that statins may attenuate inflammation, oxidative stress, coagulation, platelet aggregation, and improve insulin resistance, fibrinolysis and endothelial functions and help to prevent thrombosis, restenosis or organ transplantation rejection. Statins may affect the intracellular prenylation of proteins,

which modulate the activity of small-GTP binding proteins. Statins have an excellent safety profile and seldom cause adverse effects. Increasing evidence suggests that statins are the current treatment of choice to prevent vascular complications in diabetic patients with hypercholesterolemia (Ludwig and Shen, 2006). However, any individual with changes that suggest severe coronary syndrome should be taken in consideration for myocardial revascularization (Legrand and Legrand, 2005). Individuals with diabetes type 1 are at a very high risk of coronary artery disease and cardiovascular death in relation to the regu! lar population (Laing et al., 2003). In Laing et al, study it has been found that although ischemic heart disease is the predominant cause of mortality in older people with diabetes, age-specific mortality rates have not been published for patients with Type 1 diabetes. The Diabetes UK cohort now has sufficient follow-up to report all heart disease, and specifically ischemic heart disease, mortality rates by age. A cohort of 23,751 patients with insulin-treated diabetes, diagnosed under the age of 30 years and from throughout the United Kingdom, was identified during the period 1972 to 1993 and followed for mortality until December 2000. Age- and sex-specific heart disease mortality rates and standardized mortality ratios were calculated. There were 1437 deaths during the follow-up, 536 from cardiovascular disease, and of those, 369 from ischemic heart disease. At all ages the ischemic heart disease mortality rates in the cohort were higher than in the general population. M! ortality rates within the cohort were similar for men and women under the age of 40. The standardized mortality ratios were higher in women than men at all ages, and in women were 44.8 at ages 20-29 and 41.6 at ages 30-39. The risk of mortality from ischemic heart disease is exceptionally

high in young adult women with Type 1 diabetes, with rates similar to those in men with Type 1 diabetes under the age of 40. These observations emphasize the need to identify and treat coronary risk factors in these young patients (Laing et al., 2003). In Kretowski et al, the purpose of the study was to evaluate the association of apolipoprotein A-IV (APOA4) polymorphisms with coronary artery calcification (CAC) progression, a marker of subclinical atherosclerosis. Two previously well-studied functional APOA4 polymorphisms resulting in the substitution of the amino acid Thr for Ser at codon 347 and Gln for His at codon 360 were genotyped in 634 subjects with type 1 diabetes and 739 non-diabetic control subjects, the participants of the prospective Coronary Ar! tery Calcification in Type 1 Diabetes (CACTI) study. The His360 allele was associated with a significantly higher risk of CAC progression among patients with type 1 diabetes (33.7 vs. 21.2%, p=0.014), but not in the control subjects (14.1 vs. 11.1%, p=0.42). Logistic regression analysis confirmed that the presence of the APOA4 His360 allele predicts an increased risk of progression of coronary atherosclerosis in adults with type 1 diabetes of long duration (odds ratio = 3.3, p=0.003 after adjustment for covariates associated with CAD risk). This is the first report suggesting an association between the APOA4 Gln360His polymorphism and risk of CAC progression in subjects with type 1 diabetes. Additional studies are needed to explore potential interactions between APOA4 genotypes and metabolic/oxidative stress components of the diabetic milieu leading to rapid progression of atherosclerosis. It is not known very much about the potential that causes accelerated coronary artery! atherosclerosis, whereas chronic hyperglycemia and insulin resistance explains a part of this excess of coronary artery disease in type

1 diabetes (Kretowski et al., 2006).

One of the main susceptible and precise indications of coronary atherosclerotic plaque burden is coronary artery calcification and which had shown to expect coronary proceedings (Raggi et al., 2003; Pletcher et al., 2004). In the study of Raggi et al, an observational study relating the occurrence of acute myocardial infarction (MI) to coronary artery calcium progression in 817 asymptomatic subjects referred for sequential electron beam tomographic imaging (average interval 2.2 +/- 1.3 years). A calcium volume score (CVS) was used for plaque quantification. The yearly mean absolute and percent CVS changes in the 45 patients who had a MI were 147 +/- 152 and 47 +/- 50%, respectively, compared with 63 +/- 128 and 26 +/- 32%, respectively ($p < 0.001$, $p = 0.01$), in patients without events (Raggi et al., 2003). In patients with diabetes, especially women, the frequency of coronary calcification is higher (Colhoun et al., 2000). In the study of Colhoun et al, the objective of ! the study was to examine whether the gender difference in coronary artery calcification, a measure of atherosclerotic plaque burden, is lost in type 1 diabetic patients, and whether abnormalities in established coronary heart disease risk factors explain this. Type 1 diabetes abolishes the gender difference in coronary heart disease mortality because it is associated with a greater elevation of coronary disease risk in women than men. Coronary artery calcification and coronary risk factors were compared in 199 type 1 diabetic patients and 201 nondiabetic participants of similar age (30 to 55 years) and gender (50% female) distribution. Only one subject had a history of coronary disease. Calcification was measured with electron beam computed tomography. In nondiabetic participants there was a large gender difference in

calcification prevalence (men 54%, women 21%, odds ratio 4.5, $p < 0.001$), half of which was explained by established risk factors (odds ratio after adjustment! = 2.2). Diabetes was associated with a greatly increased prevalence of calcification in women (47%), but not men (52%), so that the gender difference in calcification was lost ($p = 0.002$ for the greater effect of diabetes on calcification in women than men). On adjustment for risk factors, diabetes remained associated with a threefold higher odds ratio of calcification in women than men ($p = 0.02$). In type 1 diabetes coronary artery calcification is greatly increased in women and the gender difference in calcification is lost (Colhoun et al., 2000).

Coronary calcification has been discovered to separately predict myocardial infarction or disruptive coronary artery disease in diabetics (Matthews et al., 2006). In the study of Matthews et al, it has been shown that a longstanding hypothesis is that individuals who exhibit large increases in blood pressure during psychological stress are at risk for atherosclerosis. It was tested whether blood pressure changes during psychological stress predict subsequent coronary calcification (CaC) in young healthy adults. It was evaluated 2816 healthy black and white women, 20 to 35 years of age, from the Coronary Artery Risk Development in Young Adults Study, who were not using medication for hypertension or diabetes in 1987-1988. Participants completed video game and star tracing tasks while their blood pressure was recorded. Thirteen years later (2000-2001), they completed computed tomography measures of CaC. Overall 9.3% (261 of 2816) had CaC present at follow-up. Each 10 mm Hg! change in systolic blood pressure during the video game was associated with a 24% increased odds of having CaC at follow-up (unadjusted odds ratio, 1.24; 95% CI, 1.06 to 1.46;

P=0.008). This association persisted after adjustment for age, race, sex, education, smoking, alcohol, smoking, daily alcohol consumption, body mass index, resting or baseline blood pressure and family history of myocardial infarction (odds ratio, 1.31; 95% CI, 1.08 to 1.58; P=0.006). Blood pressure changes during the star tracing task were not associated with subsequent CaC. Blood pressure changes during a video game predicted the presence of CaC 13 years later. This is the first study that reports blood pressure reactivity to a stressor being related to calcification in the coronary arteries (Matthews et al., 2006).

Peripheral Vascular Disease and Diabetes

Hypertension normally coexists through diabetes, and this considerably enlarges the probability of vascular complication (Sowers et al., 2001). In the review of Sowers et al, it is stated that hypertension is approximately twice as frequent in patients with diabetes compared with patients without the disease. Conversely, recent data suggest that hypertensive persons are more predisposed to the development of diabetes than are normotensive persons (Sowers et al., 2001). The beginning characteristic of vascular complications in diabetics consists of endothelial dysfunction with moderated endothelium-dependent vasodilatation, increased vascular permeability and the progress of prothrombotic symptoms. Short-term improvement in glycemic control does not appear to reduce endothelial activation (Bagg et al., 2001). In the study of Bagg et al, the aims of this study were to elucidate the factors that contribute to endothelial activation and fibrinolytic abn! ormalities in patients with poorly controlled type 2 diabetes and to

determine whether improved glycemic control reduces endothelial activation. Adhesion molecules [E-selectin, intracellular adhesion molecule-1 (ICAM-1), and vascular cell adhesion molecule-1], von Willebrand factor, total nitric oxide (NO), endothelin-1, tissue plasminogen activator, and plasminogen activator inhibitor-1 were measured in 43 type 2 diabetic subjects with hemoglobin A1c of 9.0% or more at baseline (compared with 21 healthy controls) who after 20 wk had been randomized to either improved (IC) or usual (UC) glycemic control. At baseline, type 2 diabetic patients had significant endothelial activation and abnormal fibrinolysis compared with control subjects. Body mass index in the diabetic patients was the only independent predictor of E-selectin ($P = 0.007$), ICAM-1 ($P = 0.01$), and NO ($P = 0.008$) concentrations, but not vascular cell adhesion molecule-1, plasminogen activator inhibitor-1, or tis! sue plasminogen activator (all $P > 0.05$). Type 2 diabetic patients with a body mass index of 28 kg/m2 or less had concentrations of E-selectin, ICAM-1, endothelin-1, and NO similar to those in healthy controls. After 20 wk, hemoglobin A1c was significantly lower in IC vs. UC (IC, 8.02 +/- 0.25%; UC, 10.23 +/- 0.23%; $P < 0.0001$), but there were no significant changes in markers of endothelial activation or indexes of fibrinolysis. Obesity appears to be the most important predictor of endothelial activation in patients with type 2 diabetes (Bagg et al., 2001).

Hyperglycemia leads to increased oxidative stress and monocyte and endothelial cell dysfunction (Jain et al., 2006). In the study of Yung et al, it is found that reactive oxygen species as well have different physiological and pathophysiological contacts on vascular cells and have a part in vascular dysfunction and transforming via oxidative damage by decreasing the bioavailability of NO, damaging endothelium dependent anoikis, accelerating endothelial cell migration, and initiating adhesion molecules and

inflammatory reaction, directing to endothelial dysfunction, an early episode succeeding regarding hypertension and atherosclerosis. In addition, cellular events underlying these developments include modification in vascular smooth muscle cell growth, apoptosis, cell movement, inflammation, and vasoconstriction (Yung et al., 2006). Vascular endothelial cells have a number of physiological events that are necessary for the regular role of the cardiovascular system ! (Fadini et al., 2006). In the study of Fadini et al, the objective of the study was to establish whether number and function of endothelial progenitor cells EPCs correlate with peripheral arterial disease (PAD) severity in type 2 diabetic patients. EPCs were defined by the expression of CD34, CD133 and KDR, and quantified by flow cytometry in 127 diabetic patients with and without PAD. PAD severity has been assessed as carotid atherosclerosis and clinical stage of leg atherosclerosis obliterans. Diabetic patients with PAD displayed a significant 53% reduction in circulating EPCs versus non-PAD patients, and EPC levels were negatively correlated with the degree of carotid stenosis and the stage of leg claudication. Moreover, the clonogenic and adhesion capacity of cultured EPCs were significantly lower in diabetic patients with PAD versus patients without. This study demonstrates that EPC decrease is related to PAD severity and that EPC function is altered in diabetic subjec! ts with PAD, strengthening the pathogenetic role of EPC deregulation in diabetic vasculopathy. EPC count may be considered a novel biological marker of peripheral atherosclerosis in diabetes (Fadini et al., 2006). In the study of Haidara et al, vascular endothelial cells have several physiological actions that are essential for the normal function of the cardiovascular system. The physiological events consist of the production of nitric oxide that controls vasodilatation, anticoagulation, leukocyte adhesion, smooth muscle proliferation, and the

antioxidative capacity of endothelial cells (Haidara et al., 2006). Furthermore, oxidative stress produces damage in a cell (Sampson et al., 2006). In the study of Sampson et al, it is shown that telomeres are DNA sequences necessary for DNA replication, which shorten at cell division at a rate related to levels of oxidative stress. Once shortened to a critical length, cells are triggered into replicative senescence. Type 2 diabetes is associated with oxidative DNA damage, and it is hypothesized that te! lomere shortening would characterize type 2 diabetes. Twenty-one male type 2 diabetic subjects (mean age 61.2 years, mean HbA (1c) 7.9%) were studied and selected to limit confounding effects on telomere length and 29 matched control subjects. Telomere length was measured in peripheral venous monocyte and T-cells by fluorescent in situ hybridization and oxidative DNA damage by flow cytometry of oxidized DNA bases. Peripheral insulin resistance and high-sensitivity C-reactive protein (hsCRP) were measured. Mean monocyte telomere length in the diabetic group was highly significantly lower than in control subjects (4.0 [1.1] vs. 5.5 [1.1]; $P < 0.0001$), without significant differences in lymphocyte telomere length. There was a trend toward increased oxidative DNA damage in all diabetes cell types examined and a significant inverse relationship between oxidative DNA damage and telomere length ($r = -0.55$; $P = 0.018$) in the diabetic group. Telomere length was unrelated to plasma C! RP concentration or insulin resistance. Monocyte telomere shortening in type 2 diabetes could be due to increased oxidative DNA damage to monocyte precursors during cell division. This data suggests that monocytes adhering to vascular endothelium and entering the vessel wall in type 2 diabetes are from a population with shorter telomeres and at increased risk of replicative senescence within vascular plaque (Sampson et al., 2006).

Cerebrovascular Disease and Diabetes

Diabetes mellitus is familiar disease distinguished by adjustments in microvessels in multiple tissues, ensuing in retinopathy, nephropathy and neuropathy (Gigante, 2006). In the study of Gigante et al, it was shown that diabetic nephropathy is a microvascular complication, as well as retinopathy and neuropathy of type 1 and type 2 diabetes mellitus. The pathogenesis directly correlates with hyperglycemia. The direct glucose toxicity, hypercoagulability, oxidative stress and endothelial dysfunctions play a role. Strict glycemic control with HbA1c levels <7% for 10 yrs is associated with a 25% microvascular end point reduction. Patients underwent pancreas transplantation, and after 10 yrs the present nephropathy and functional and structural abnormalities have regressed. Diabetic nephropathy alters the pharmacokinetic profile of almost all oral anti-diabetic agents, as well as insulin metabolism; therefore, it is imperative to determine creatin! ine clearance. Renal failure requires insulin therapy. Incipient diabetic nephropathy allows very limited indications to oral anti-diabetic agents because of the altered pharmacokinetic profile and side effects (hypoglycemia with secretagogues agents, lactic-acidosis with metformin and other biguanides, and hydric retention and weight gain with thiazolidinediones). Moreover, oral anti-diabetic agents reduce the percentage of HbA1c by no more than 1.5%. Insulin therapy is preferred, with a dose reduction when creatinine clearance is <60 mL/m. To control better the post-prandial glycemic peak, it is useful to use bolus insulin analogues at each meal and basal intermediate-

acting insulin at bedtime to mimic the natural insulin patterns as far as possible (Gigante, 2006).

Current studies have accentuated that reactive oxygen species are very much related with the progress of diabetes-specific complications (Niiya et al., 2006). In Niiya et al, study it was shown that there is accumulating evidence that advanced glycation end products (AGEs) are relevant to the formation of vascular complications in diabetes mellitus. The aim of this study was to investigate whether AGEs have a significant effect on tissue factor (TF) expression in brain microvascular endothelial cells compared with that in other arterial endothelial cells. Cultured bovine brain microvascular endothelial cells (BBMECs) and aortic endothelial cells (BAECs) were incubated in medium containing glyceraldehydes-derived AGE (glycer-AGE). TF mRNA expression, protein expression, and activity were measured at multiple time points after glycer-AGE incubation. Participation of reactive oxygen species (ROS) in the effect of glycer-AGE on TF expression was investigated by treatment with a! free radical scavenger, edaravone, and intracellular ROS measurements with dihydroethidium (DHE). Basic TF mRNA expression was greater in BBMECs than in BAECs. Glycer-AGE significantly upregulated TF mRNA expression in both cells, and the upregulation was more prominent in BBMECs than in BAECs. TF protein expression and activity were also upregulated with a pattern of being greater in BBMECs than in BAECs. Edaravone significantly attenuated the AGE-induced upregulation of TF mRNA expression, protein expression, and activity. Intracellular ROS levels measured with DHE-stained fluorescent intensity were significantly upregulated by glycer-AGE with a pattern of being greater in BBMECs

than in BAECs. AGE-induced ROS upregulation was attenuated by edaravone like AGE-induced TF upregulation. These results suggest that brain microvascular endothelial cells are more susceptible to AGE-induced TF upregulation than aortic endothelial cells, and that this susceptibility is associated! with levels of intracellular ROS (Niiya et al., 2006).

Reactive oxygen species can produce DNA oxidation by diverse procedures, such as through hydroxyl radicals, which are consequential from the lessening of H_2O_2 or through reactive nitrogen intermediates for instance peroxynitrate (Low et al., 1997). In the study made by Low et al, it can be found that antioxidant enzymes are reduced in peripheral nerves and are further reduced in diabetic nerves. That lipid peroxidation will cause neuropathy is supported by evidence of the development of neuropathy de novo when normal nerves are rendered alpha-tocopherol deficient and by the augmentation of the conduction deficit in diabetic nerves subjected to this insult. Oxidative stress appears to be primarily due to the processes of nerve ischemia and hyperglycemia auto-oxidation. The indexes of oxidative stress include an increase in nerve, dorsal root, and sympathetic ganglia lipid hydroperoxides and conjugated dienes. The most reliable and sensitive index, howe! ver, is a reduction in reduced glutathione. Experimental diabetic neuropathy results in myelinopathy of dorsal roots and a vacuolar neuropathy of dorsal root ganglion. The vacuoles are mitochondrial; it was posit that lipid peroxidation causes mitochondrial DNA mutations that increase reduced oxygen species, causing further damage to mitochondrial respiratory chain and function and resulting in a sensory neuropathy. Alpha-lipoic acid is a potent antioxidant that prevents lipid peroxidation in vitro and

in vivo. It was evaluated the efficacy of the drug in doses of 20, 50, and 100 mg/kg administered intraperitoneally in preventing the biochemical, electrophysiological, and nerve blood flow deficits in the peripheral nerves of experimental diabetic neuropathy. Alpha-lipoic acid dose- and time-dependently prevented the deficits in nerve conduction and nerve blood flow and biochemical abnormalities (reductions in reduced glutathione and lipid peroxidation). The nerve blood flow! deficit was 50% ($P < 0.001$). Supplementation dose-dependently prevented the deficit; at the highest concentration, nerve blood flow was not different from that of control nerves. Digital nerve conduction underwent a dose-dependent improvement at 1 month ($P < 0.05$). By 3 months, all treated groups had lost their deficit. The antioxidant drug is potentially efficacious for human diabetic sensory neuropathy (Low et al., 1997).

A connecting relationship among oxidative stress and diabetic nephropathy has been found by various observations, as well as a result that formation of 8-hydroxyl-2' deoxyguanosine, an oxygen radical stimulated by alteration of a purine remains in DNA, was thoroughly connected to the development of diabetic nephropathy (Ha et al., 1994). In Ha et al, study, 8-Hydroxydeoxyguanosine (8-OHdG), an oxygen radical induced modification of purine residue in DNA, was measured in the liver, pancreas, and kidney of streptozotocin-induced diabetic rats (STZR) exhibiting microalbuminuria. At 4 weeks after the injection of streptozotocin (50 mg/kg, i.v.), the rate of urinary albumin excretion was 0.5 +/- 0.1 and 2.0 +/- 0.2 mg/24 h in age-matched control rats (CR) and STZR, respectively. Compared to CR, STZR also showed a significantly increased level of 8-OHdG in the kidney but not the liver and pancreas. Amounts of 8-OHdG/10(5) dG for CR and STZR were 3.4 +/-

0.3 and 5.1 +/- 0.2! for renal cortices, and 4.1 +/- 0.2 and 20.0 +/- 3.7 for renal papillae. Daily injection of insulin (2 U, SC) starting on the third day after streptozotocin treatment significantly reduced both urinary albumin excretion and papillary 8-OHdG formation, which suggests that these are associated with the diabetic state induced by streptozotocin rather than a direct nephrotoxic effect of the drug. This study suggests that formation of 8-OHdG and, therefore, oxidative damage is closely related in the process of diabetic nephropathy (Ha et al., 1994).

Therapy by means of a diversity of antioxidants compounds for instance vitamin E and others, concluded in a considerable decrease in symptoms of retinopathy and neuropathy in people and investigational diabetes (Ndahimana et al., 1996). In the study of Ndahimana et al, some biologic parameters involved in cell defense against oxygen radicals (plasmatic vitamins C and E, erythrocyte glutathione peroxidase, glutathione reductase and superoxide dismutase) were measured in single blood samples from 119 diabetic infants, adolescents and young adults. Data were studied in relation to residual insulin secretion determined by C peptide, level of metabolic control appreciated by glycosylated hemoglobin, lipid abnormalities and subclinical complications (retinopathy, neuropathy and nephropathy). There was no change in antioxidant parameters with insulin secretion. Patients with poor glycemic control and high plasma lipids had higher levels of plasma vitamin E. Patients with nephropath! y had lower plasma vitamin C levels and those with neuropathy showed lower erythrocyte glutathione peroxidase activity. Plasma vitamin C concentrations and erythrocyte glutathione reductase activities were negatively correlated with the age of the patients and the duration of the disease. Higher transport capacity of

vitamin E probably explains the elevated levels of vitamin E observed in patients with high lipid levels and long lasting illness. The lower levels of vitamin C in the presence of nephropathy may be due to an increased renal excretion of this vitamin. The reduction of glutathione peroxidase, glutathione reductase activities and vitamin C levels confirms the existence of an oxidative stress in type I diabetes (Ndahimana et al., 1996).

Though, the part that oxidative stress plays in the brain continues to be undefined. A significant factor that contributes to the modification in the central nervous system is the blood-brain barrier (Farr et al., 2006). In the study of Farr it is shown that leptin is a peptide hormone secreted by adipose tissue. Studies have shown that leptin crosses the blood-brain barrier (BBB) by a saturable transport system where it acts within the hypothalamus to regulate food intake and energy expenditure. Leptin also acts in the hippocampus where it facilitates the induction of long-term potentiation and enhances NMDA receptor-mediated transmission. This suggests that leptin plays a role in learning and memory. Obese mice and rats, which have leptin receptor deficiency, have impaired spatial learning. In disease states such as diabetes, humans and animals develop leptin resistance at the BBB. This suggests that low leptin levels in the brain may be involved in cognitive deficits asso! ciated with diabetes. In the current study, the effects of leptin on post-training memory processing in CD-1 mice were examined. Mice were trained in T-maze footshock avoidance and step down inhibitory avoidance. Immediately after training, mice received bilateral injections of leptin into the hippocampus. Retention was tested 1 week later in the T-maze and 1 day later in step down inhibitory avoidance. Leptin

administration improved retention of T-maze footshock avoidance and step down inhibitory avoidance. Leptin administered 24 h after T-maze training did not improve retention when tested 1 week after training. SAMP8 mice (a strain of mice with elevated amyloid-beta protein) at 12 months of age have elevated amyloid-beta protein and impaired learning and memory. It was examined the effect of leptin on memory processing in the hippocampus of 4 and 12 months old SAMP8 mice. Leptin improved retention in both 4 and 12 months old SAMP8 mice; 12 month SAMP8 mice requ! ired a lower dose to improve memory compared to 4 months SAMP8 mice. The current results indicate that leptin in the hippocampus is involved in memory processing and suggests that low levels of leptin may be involved in cognitive deficits seen in disease states where leptin transport into the CNS is compromised (Farr et al., 2006).

Diabetes is connected with modifications in both the barrier and transport functions of the cerebral microvessels (Mooradian, 1997). In the review of Mooradian et al, it is specified that structural changes in cerebral microvessels may account for some of the observed changes. Additional mechanisms include alterations in homodynamic variables such as arteriovenous shunting, changes in biophysical properties and biochemical compositions of the endothelial cells including changes in lipid fluidity and composition, and alterations of neurotransmitter activity in the cerebral microvessels, notably altered beta adrenergic neurotransmission. These observations indicate that the CNS is not immune against the microangiopathic complications commonly found in various tissues of diabetic animals (Mooradian, 1997). Structural modifications in cerebral microvessels may be responsible for

couple of the changes that were noticed in diabetes.

It is thought that hyperglycemia causes oxidative injury in cerebral microvessels which then trigger the blood-brain barrier to degenerate, resulting in diabetic cerebral brain injury (Packer et al., 1997). In the review of Packer et al, it is specified that reactive oxygen species is thought to be involved in a number of types of acute and chronic pathologic conditions in the brain and neural tissue. The metabolic antioxidant alpha-lipoate (thioctic acid, 1, 2-dithiolane-3-pentanoic acid; 1, 2-dithiolane-3 valeric acid; and 6, 8-dithiooctanoic acid) is a low molecular weight substance that is absorbed from the diet and crosses the blood-brain barrier. Alpha-Lipoate is taken up and reduced in cells and tissues to dihydrolipoate, which is also exported to the extracellular medium; hence, protection is afforded to both intracellular and extracellular environments. Both alpha-lipoate and especially dihydrolipoate have been shown to be potent antioxidants, to regenerate through ! redox cycling other antioxidants like vitamin C and vitamin E, and to raise intracellular glutathione levels. Thus, it would seem an ideal substance in the treatment of oxidative brain and neural disorders involving free radical processes. Examination of current research reveals protective effects of these compounds in cerebral ischemia-reperfusion, excitotoxic amino acid brain injury, mitochondrial dysfunction, diabetes and diabetic neuropathy, inborn errors of metabolism, and other causes of acute or chronic damage to brain or neural tissue. Very few neuropharmacological intervention strategies are currently available for the treatment of stroke and numerous other brain disorders involving free radical injury. It is propose that the various metabolic antioxidant properties of alpha-lipoate relate to its

possible therapeutic roles in a variety of brain and neuronal tissue pathologies: thiols are central to antioxidant defense in brain and other tissues. The most important ! thiol antioxidant, glutathione, cannot be directly administered, whereas alpha-lipoic acid can. In vitro, animal, and preliminary human studies indicate that alpha-lipoate may be effective in numerous neurodegenerative disorders (Packer et al., 1997).

It is acknowledged that amplified vascular permeability is associated to diabetic retinopathy (El-Remessy et al., 2006), even though disapproval exists whether blood-brain barrier permeability is high in the cerebral microvessels of diabetic animals (Mooradian, 1997). In the study of El-Remessy et al, diabetic retinopathy is characterized by blood-retinal barrier (BRB) breakdown and neurotoxicity. These pathologies have been associated with oxidative stress and proinflammatory cytokines, which may operate by activating their downstream target p38 MAP kinase. In the present study, the protective effects of a nonpsychotropic cannabinoid, cannabidiol (CBD), were examined in streptozotocin-induced diabetic rats after 1, 2, or 4 weeks. Retinal cell death was determined by terminal dUTP nick-end labeling assay; BRB function by quantifying extravasations of bovine serum albumin-fluorescein; and oxidative stress by assays for lipid peroxidation, dichlorofluorescein fluorescence, and! tyrosine nitration. Experimental diabetes induced significant increases in oxidative stress, retinal neuronal cell death, and vascular permeability. These effects were associated with increased levels of tumor necrosis factor-alpha, vascular endothelial growth factor, and intercellular adhesion molecule-1 and activation of p38 MAP kinase, as assessed by enzyme-linked immunosorbent assay, immunohistochemistry, and/or Western blot. CBD treatment

significantly reduced oxidative stress; decreased the levels of tumor necrosis factor-alpha, vascular endothelial growth factor, and intercellular adhesion molecule-1; and prevented retinal cell death and vascular hyperpermeability in the diabetic retina. Consistent with these effects, CBD treatment also significantly inhibited p38 MAP kinase in the diabetic retina. These results demonstrate that CBD treatment reduces neurotoxicity, inflammation, and BRB breakdown in diabetic animals through activities that may involve inhibition of ! p38 MAP kinase (El-Remessy et al., 2006). A study made by Tapp et al, shows progression and risk factors for diabetic retinopathy in the multiethnic population of Mauritius. A longitudinal, population-based study was conducted in Mauritius, during 1987, 1992 and 1998. Participants identified through the study as having diabetes and one in four participants with impaired glucose tolerance (IGT) underwent complications screening in 1992 and 1998. Retinal photographs were taken using a TRC-50VT retinal camera in three fields of the right eye. Photographs were graded according to a simplified version of the Wisconsin grading system. The 6-year incidence of diabetic retinopathy was 23.8% (sight-threatening in 0.4%). Among those with known diabetes mellitus (KDM) and free of retinopathy at baseline the incidence of non-proliferative diabetic retinopathy (NPDR) was 29.2% and proliferative diabetic retinopathy (PDR) was 1.0%. Among those with newly diagnosed diabetes mellitus (NDM) at baseline the incidence of NPDR was 19.1%. Independent risk factors ! for retinopathy using the baseline population characteristics were duration of diabetes and fasting plasma glucose. This is one of the few recent population-based studies of diabetic retinopathy undertaken in a developing nation. The incidence of retinopathy in Mauritius was

high among those with NDM at baseline, with one in five developing retinopathy over 6 years (Tapp et al., 2006).

OXIDATIVE STRESS AND NEUROPATHY COMPLICATIONS

In the central nervous system, oxidative stress signifies an important pathway that leads to the damage of both neuronal and vascular cells (Root-Bernstein et al., 2002). In the study of Root-Bernstein et al, vitamin C exists in two major forms. The charged form, ascorbic acid (AA), is taken up into cells via sodium-dependent facilitated transport. The uncharged form, dehydroascorbate (DHA), enters cells via glucose transporters (GLUT) and is then converted back to AA within these cells. Cell types such as certain endothelial and epithelial cells as well as neurons that are particularly prone to damage during diabetes tend to be those that appear to be dependent on GLUT transport of DHA rather than sodium-dependent AA uptake. We hypothesize that diabetic neuropathies, nephropathies and retinopathies develop in part by exclusion of DHA uptake by GLUT transporters when blood glucose levels rise above normal. AA plays a central role in the antioxidant ! defense system. Exclusion of DHA from cells by hyperglycemia would deprive the cells of the central antioxidant, worsening the hyperglycemia-induced oxidative stress level. Moreover, AA participates in many cellular oxidation-reduction reactions including hydroxylation of polypeptide lysine and proline residues and dopamine that are required for collagen production and metabolism and storage of catecholamines in neurons. Increase in the oxidative stress level and metabolic perturbations can be expected in any tissue or cell type that relies exclusively or mainly

on GLUT for co-transport of glucose and DHA including neurons, epithelial cells, and vascular tissues. On the other hand, since DHA represents a significant proportion of total serum ascorbate, by increasing total plasma ascorbate concentrations during hyperglycemia, it should be possible to correct the increase in the oxidative stress level and metabolic perturbations, thereby sparing diabetic patients many of thei! r complications (Root-Bernstein et al., 2002).

Diabetic neuropathy most likely comes from a mixture of micro vascular along with neuronal deficits, and oxidative stress participates in the breakdown of neuronal phenotype in experimental diabetic neuropathy (Sima et al., 2004). In Sima et al, study it is compared the effects of streptozotocin-induced diabetes in rats with those of two pro-oxidant interventions; a diet deficient in vitamin E and treatment with primaquine. Measurements were made by the classic motor and sensory conduction velocity deficits and by indicators of the breakdown of small fiber phenotype i.e., sciatic nerve content of nerve growth factor and the neuropeptides, substance P and neuropeptide Y. As with diabetes, the pro-oxidant interventions decreased conduction velocities (though the effect of vitamin E deficiency was not significant), the sciatic nerve content of nerve growth factor and the neuropeptides (all percentages refer to the mean value for the appropriate control groups). In diabetes, ne! rve growth factor was depleted to 50% in the control rats ($p < 0.05$); oxidative stress depleted nerve growth factor to 64% (primaquine; $p < 0.05$) and 81% (vitamin E deficient; not significant) of controls. Substance P was depleted to 51% in the control rats ($p < 0.01$) with depletions to 74% and 72% (both $p < 0.01$) by oxidative stress; equivalent depletions for neuropeptide Y were 38% controls in diabetes ($p < 0.001$) and 67% (primaquine; $p < 0.001$) and 74% (vitamin E deficient; $p < 0.05$) for

oxidative stress. The relative magnitudes of these changes suggest an effect in diabetes of oxidative stress, coupled with some other cellular event(s). This is supported by the effects of a diester of gamma-linolenic acid and alpha-lipoic acid, which completely prevented the effects on the pro-oxidant interventions on conduction velocity, nerve growth factor and neuropeptide contents, but was only partially preventative in diabetes (Sima et al., 2004). Oxidative stress has a considerabl! e involvement with these deficits because they might be the consequence of hyperglycemia (Hockett et al., 2004). In Hockett et al, study, infants of diabetic mothers have an increased frequency of congenital anomalies, including CNS malformations. Fetal hyperglycemia may promote such damage via oxidative stress. Postmortem studies have shown that fetal hyperglycemia associated with maternal diabetes results in islet cell hyperplasia. Islet cell hyperplasia may correlate with the presence of oxidative stress injury in the CNS because of hyperglycemia and related metabolic derangement. This study examines 3-nitrotyrosine immunoreactivity as a marker of oxidative stress in the brains of fetuses stratified by the presence or absence of islet cell hyperplasia and CNS developmental anomalies. Fetuses with both islet cell hyperplasia and CNS developmental anomalies showed a 1.8-fold increase in semiquantitatively scored 3-nitrotyrosine immunostaining compared to negative controls. Fetuses with islet cell hyperplasia but no CNS anomalies demonstrated ! a 1.6-fold increase. Comparison between fetuses with islet cell hyperplasia, which was stratified by presence, or absence of CNS anomalies were not statistically different but did show more intense staining in those with CNS malformations. These results support the contention that hyperglycemia may contribute to CNS malformation via oxidative stress (Hockett et al., 2004).

Lowering glucose during diabetes also produces apoptosis of peripheral neurons throughout a mechanism that at least includes oxidative stress to some extent (Honma et al., 2003). In Honma et al, study, the aim was to understand better the mechanisms underlying peripheral neuropathy with diabetes mellitus and to test the hypothesis that acute lowering of glucose levels induces apoptosis in hypoxic neurons. It was used rat dissociated dorsal root ganglion (DRG) neurons incubated in a medium high in glucose concentration (700 mg%) and room air (PO2 150 torr). After 5 days, DRG neurons were placed in hypoxic conditions (PO2 7.6 torr) with a normal-glucose (100 mg%) or high-glucose (700 mg%) medium containing 3 or 100 ng/mL of nerve growth factor. Acute lowering of glucose levels under hypoxic conditions led to apoptosis of DRG neurons. Bis-benzimide staining for nuclear fragmentation, electron microscopy, DNA laddering, and TUNEL staining demonstrated apoptosis. Caspase 3 im! munocytochemistry and inhibition of neuronal death by the caspase inhibitor z-VAD-fmk (100 microM) confirmed that death was apoptotic. Hypoxia-induced death was decreased when DRG neurons were maintained in high-glucose medium, suggesting that high levels of substrate protected against hypoxia. Apoptosis was completely prevented by increasing the concentration of nerve growth factor from 3 to 100 ng/mL and was partially prevented by the addition of the antioxidant alpha-lipoic acid (500 microM) (Honma et al., 2003).

Oxidative Stress and the Central Nervous System

Oxidative stress takes place within the brain while the production of reactive

oxygen species take precedence over the capability of the endogenous antioxidant structure to eliminate excess reactive oxygen species afterwards directing to cellular injury. Cellular characteristics of the brain indicate that this process is very delicate to oxidative stress. The brain, for instance, needs a very high amount of oxygen in order to work. It necessitates about 20% of oxygen for the brain located in the entire body. Furthermore, the brain tissue includes a large amount of unsaturated fatty acids, which are metabolized through oxygen free radicals and therefore, the brain includes high percentages of iron, which have been connected to free radical damage (Miyajima et al., 2003). In the study of Miyajima et al, ceruloplasmin, a multi-copper ferroxidase that affects the distribution of tissue iron, has antioxidant effects through the oxidation of ferrous iron to ferric iron. Acerulopl! asminemia is an inherited disorder of iron metabolism due to the complete lack of ceruloplasmin ferroxidase activity caused by mutations in the ceruloplasmin gene. It is characterized by iron accumulation in the brain as well as visceral organs. Clinically, the disease consists of the triad of retinal degeneration, diabetes mellitus, and neurological disease, which include ataxia, involuntary movements, and dementia. These symptoms reflect the sites of iron deposition. The unique involvement of the central nervous system distinguishes aceruloplasminemia from other inherited and acquired iron storage disorders. Twenty-one mutations in the ceruloplasmin gene have been reported in 24 families worldwide. In Japan, the incidence was estimated to be approximately one per 2,000,000 in the case of non-consanguineous marriages. Excess iron functions as a potent catalyst of biologic oxidation. Previously it was shown that an increased iron concentration is associated with increased l! evels of lipid peroxidation in the serum, cerebrospinal fluid, and erythrocyte

membranes. The levels of malondialdehyde and 4-hydroxynonenals, indicators of lipid peroxidation, were also elevated in the basal ganglia and cerebral cortex. Positron emission tomography shown diminished brain metabolism of glucose and oxygen. Enzyme activities in the mitochondrial respiratory chain of the basal ganglia were reduced to approximate 45% and 42%, respectively, for complexes I and IV. These findings suggest that iron-mediated free radicals causes neuronal cell damage through lipid peroxidation and mitochondrial dysfunction in aceruloplasminemia brains (Miyajima et al., 2003). The brain may also endure from the incorrect resistances anti oxidative stress. Oxidative stress corresponds to an important pathway that leads to the damage of neuronal along with vascular cells in central nervous system.

Oxidative Stress and Alzheimer's Disease

The relationship between diabetes and dementia provokes strong disagreements because of the practical variations of the different studies about the classification of dementia. It was noticed in Alzheimer's disease, a decrease in cerebral glucose exploitation, still while corrected for brain atrophy (Ibanez et al., 1998). In the study of Ibanez et al, the aim was to determine whether the hypometabolism observed in PET images of patients with Alzheimer's disease (AD) is due entirely to brain atrophy. Numerous authors have reported reduced brain glucose metabolism in AD patients measured using PET. Actual glucose metabolic values in AD may be reduced artificially because of brain atrophy, which accentuates the partial volume effect (PVE) on data collected by PET. Using segmented MR images, it was corrected regional cerebral

metabolic rates for glucose for PVEs to evaluate the effect of atrophy on uncorrected values for brain metabolism in AD patients and healthy control ! subjects. Global glucose metabolism was reduced significantly before and after correction in AD patients compared with controls. Before PVE correction, glucose metabolic values in patients were lower than in control subjects in the inferior parietal, frontal, and lateral temporal cortex; in the posterior cingulated; and in the precuneus. These reductions remained significantly lower after PVE correction, although in the posterior cingulated the difference in metabolism between AD patients and control subjects lessened. Regional glucose metabolism of these areas with PVE correction was lower in moderately-severely demented patients than in mildly demented patients. Reduced glucose metabolism measured by PET in AD is not simply an artifact due to an increase in CSF space induced by atrophy, but reflects a true metabolic reduction per gram of tissue (Ibanez et al., 1998).

Cellular injury throughout Alzheimer disease may be the effect of both reactive oxygen species and from impaired cellular repair mechanisms after oxidative injury (Chong et al., 2005). In Chong et al, review, it has been affirmed that understanding of the complex nature of the Alzheimer disease disorder has evolved with an increased appreciation for pathways that involve the generation of reactive oxygen species and oxidative stress, apoptotic injury that leads to nuclear degradation in both neuronal and vascular populations, and the early loss of cellular membrane asymmetry that mitigates inflammation and vascular occlusion (Chong et al., 2005).

The late-onset Alzheimer disease is initiated by non-insulin dependent diabetes. The concentration of insulin found in the cerebrospinal fluid was higher in Alzheimer patients than in controls. Oxidative stress is believed to have an important responsibility

in the formation of Alzheimer disease (Maiese and Chong, 2004). In the research of Maiese et al, oxidative stress precipitates both nuclear DNA degradation and membrane phosphatidylserine exposure in neuronal and vascular cells to promote loss of cellular integrity, microglial phagocytosis, and thrombotic destruction. In addition, critical in the ability to foster cell survival during oxidative stress is the modulation of the metabotropic glutamate system, cell cycle regulation in post-mitotic neurons, and control of GSK-3beta activity and presenilin integrity (Maiese and Chong, 2004).

Oxidative stress in relationship to Alzheimer disease is reliant on a few indications that are the products of oxidative stress for example, lipids, protein and DNA. These have been reported in patients with Alzheimer disease. Many people believe that Alzheimer disease is diabetes type 3 (Steen et al., 2005). In the study of Steen et al, it was studied the neurodegeneration that occurs in sporadic Alzheimer's disease (AD) is consistently associated with a number of characteristic histopathological, molecular, and biochemical abnormalities, including cell loss, abundant neurofibrillary tangles and dystrophic neurites, amyloid-beta deposits, increased activation of pro-death genes and signaling pathways, impaired energy metabolism/mitochondrial function, and evidence of chronic oxidative stress. The general inability to convincingly link these phenomena has resulted in the emergence and propagation of various heavily debated theories that focus on the role of one particular el! ement in the pathogenesis of all other abnormalities. However, the accumulating evidence that reduced glucose utilization and deficient energy metabolism occur early in the course of disease, suggests a role for impaired insulin signaling in the pathogenesis of AD. The present work demonstrates extensive

abnormalities in insulin and insulin-like growth factor type I and II (IGF-I and IGF-II) signaling mechanisms in brains with AD, and shows that while each of the corresponding growth factors is normally made in central nervous system (CNS) neurons, the expression levels are markedly reduced in AD. These abnormalities were associated with reduced levels of insulin receptor substrate (IRS) mRNA, tau mRNA, IRS-associated phosphotidylinositol 3-kinase, and phospho-Akt (activated), and increased glycogen synthase kinase-3beta activity and amyloid precursor protein mRNA expression. The strikingly reduced CNS expression of genes encoding insulin, IGF-I, and IGF-II, as well as the ! insulin and IGF-I receptors, suggests that AD may represent a neuro-endocrine disorder that resembles, yet is distinct from diabetes mellitus. Therefore, it is proposed the term, "Type 3 Diabetes" to reflect this newly identified pathogenic mechanism of neurodegeneration (Steen et al., 2005). When the brain is “fed” with insulin the brain can slow down the development of Alzheimer disease. Diabetes may increase the possibility of dementia, and there is an amplified confirmation that diabetes may perhaps be part of the cause to the development of Alzheimer disease and vascular dementia through increased oxidative stress and inflammation (Ott et al., 1999). In the study of Ott et al, the aim of the study was to determine the influence of type 2 diabetes mellitus on the risk of dementia and AD. Both dementia and diabetes are frequent disorders in elderly people. Prospective population-based cohort was studied among 6,370 elderly subjects. At baseline study participants were examined for presence of diabetes mellitus. No dement! ed participants were followed up, on average, for 2.1 years. Incident dementia was diagnosed using a three-step screening and comprehensive diagnostic workup. To complete the follow-up, medical files were studied of persons who could not be

reexamined. It was estimated relative risks with proportional hazard regression, adjusting for age, sex, and possible confounders. During the follow-up, 126 patients became demented, of whom 89 had AD. Diabetes mellitus almost doubled the risk of dementia (relative risk [RR] 1.9 [1.3 to 2.8]) and AD (RR 1.9 [1.2 to 3.1]). Patients treated with insulin were at highest risk of dementia (RR 4.3 [1.7 to 10.5]). Diabetes attributes the risk for dementia of 8.8% suggesting that diabetes may have contributed to the clinical syndrome in a substantial proportion of all dementia patients (Ott et al., 1999).

Oxidative Stress and Chronic Inflammation

Though, the reason of oxidative stress and chronic inflammation is not precisely identified it is assumed that semicarbazide-sensitive amine oxidase (SSAO) may be part of the cause to the development of Alzheimer disease (Somfai et al., 2006). In the study of Somfai et al, the aim was to investigate whether changes of soluble SSAO activity, oxidative stress and inflammation markers are related to each other in diabetes. Soluble and tissue-bound SSAO activities (from serum and aorta, respectively) were determined in streptozotocin (STZ)-induced diabetic rats without insulin treatment, receiving insulin once, or twice daily compared to control animals. After three weeks of treatment soluble and tissue-bound SSAO activities (seSSAO and aoSSAO, respectively), serum total antioxidant status (TAS), high sensitivity C-reactive protein (hsCRP), fructose amine levels and routine laboratory parameters were determined. SeSSAO activity significantly increased in the diabetic groups wit! hout treatment and receiving insulin once daily, and a marked decrease in aoSSAO activity was seen in all diabetic groups. Increased

oxidative stress was correlated with hsCRP elevation, while hsCRP and seSSAO activity were also significantly correlated. In all groups seSSAO and aoSSAO activities were in negative correlation with each other. The results supports the view that poor metabolic control leads to increased oxidative stress, which in turn may cause the elevation of hsCRP levels. Soluble SSAO on the one hand acts as an adhesion molecule--thus possibly being a factor responsible for the late complications of diabetes--and on the other hand, it may contribute to oxidative stress. The parsimonious conclusion is that there is a relation between the risk factors of AD and vascular dementia (diabetes, oxidative stress and chronic inflammation) and SSAO activity, which may originate from the vessel wall (Somfai et al., 2006). Current confirmations suggests that semicarbaz! ide-sensitive amine oxidase as well participates in having an important part in the development of late micro- and macro vascular complications of diabetes, specifically retinopathy, and atherosclerosis (Garpenstrand et al., 1999; Karadi et al., 2002). In the study of Garpenstrand et al, the aim of the study was to measure plasma semicarbazide-sensitive amine oxidase (SSAO) activities and detect retinopathy in Type 2 diabetes mellitus (DM). Cross-sectional, population-based study was made on 65 diabetes patients (61 diagnosed from the age of 30 years) with or without retinopathy as determined by fundus photography in primary care. HbA1c was analyzed by ion exchange chromatography on a Mono S for HbA1c column. SSAO activities were assayed radiometrically and formaldehyde-albumin adducts by ELISA in plasma samples from patients and 136 healthy controls. Subjects with diabetes had higher plasma SSAO activity, measured as nmol benzylamine x mlplasma(-11) x h(-1)(mean 20.6), than controls (mean 14.3), $P<0.0001$; 95% confidence interval (CI) for diff! erence 4.9-7.7.

SSAO activity was higher in patients with retinopathy (mean 23.2) than in those without (mean 18.9), P=0.012; 95% CI for difference 1.0-7.5, and related to the HbA1c value. No statistically significant relationship between diabetes duration and SSAO activity was found. With HbA1c values and insulin treatment entered into a multiple logistic regression model, SSAO activity no longer predicted retinopathy, P increasing from 0.025 to 0.17. SSAO activity and the presence of any retinopathy were unrelated to titers of antibodies against formaldehyde-treated human serum albumin. SSAO activity, earlier found to be elevated in Type 1 DM, is also elevated in Type 2 DM. The SSAO family of enzymes may be involved in the development of diabetic retinopathy, possibly by catalyzing the formation of toxic metabolites.

A potent and specific inhibitor of human SSAO might help prevent retinopathy in Type 1 and Type 2 DM (Garpenstrand et al., 1999). In the study of Karadi et al, clinical and experimental studies suggest that increased activity of semicarbazide-sensitive amine oxidase (SSAO) and the production of cytotoxic metabolites (e.g., formaldehyde and hydrogen peroxide) may play an important role in the pathogenesis of atherosclerosis. The present study was designed to assess the relationship between the increased activity of the enzyme and the severity of atherosclerosis in diabetic and control subjects. The study included 29 patients with type 2 diabetes mellitus and 25 control subjects. Human serum SSAO activity was determined by using 14C-benzylamine as substrate. Mean common carotid intima-media thickness (IMT), Crouse score and Bogousslawsky score was evaluated by color-coded, high-resolution duplex carotid sonography. Serum SSAO activity was significantly increased in patients ! with type 2 diabetes compared to controls. Carotid plaque score (Crouse score), total

cholesterol level and age-corrected intima-media thickness showed positive correlation with enzyme activity in control subjects. In patients with diabetes, serum SSAO activity correlated with the severity of carotid stenosis (Bogousslawsky score) as well as the carotid plaque score. Determination of serum SSAO activity might be a candidate biochemical marker of early atherosclerosis and diabetic macrovascular complications (Karadi et al., 2002). A report made by Ferrer has shown that tissue-bound semicarbazide-sensitive amine oxidase is overexpressed in cerebral blood vessels of individuals with Alzheimer disease (Ferrer et al., 2002), while another report made by Hernandez has shown that the action of soluble semicarbazide-sensitive amine oxidase associates with the severity of Alzheimer's disease (del Mar Hernandez et al., 2005). In the research made by Ferrer et al, semicarbazide! sensitive amine oxidase (SSAO) metabolizes oxidative deamination of primary aromatic and aliphatic amines, and, in the brain, it is selectively expressed in blood vessels. SSAO expression is examined, by immunohistochemistry with a purified polyclonal antibody to SSAO from bovine lung, in the brains of subjects with Alzheimer disease (AD; n=10), cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL; n=2), and age-matched controls (n=8). SSAO immunoreactivity restricted to meningeal and parenchymal blood vessels in control and diseased brains. Yet, a marked and selective increase in SSAO immunoreactivity occurs in association with betaA4 vascular amyloid deposits in patients with AD, and in the vicinity of the typical granular deposits in the blood vessels of gray and white matter in patients with CADASIL. Oxidative deamination of primary aromatic and aliphatic amines by SSAO produces ammonia, hydrogen peroxide and the corresponding aldehyde. Moreover, increased SSAO

immunoreactivity is associated ! with increased Cu/Zn superoxide dismutase 1 expression restricted to abnormal blood vessels in diseased brains. Therefore, it is suggested that increased SSAO expression is a source of oxidative stress in the blood vessel wall in AD and CADASIL (Ferrer et al., 2002). In the research made by del Mar Hernandez et al, semicarbazide sensitive amine oxidase (SSAO) metabolizes oxidative deamination of primary aromatic and aliphatic amines. The final products of its catalysis, ammonia, hydrogen peroxide (H2O2) and the corresponding aldehyde, may contribute to diseases involving vascular degeneration. SSAO is selectively expressed in blood vessels in the brain, but is also present in blood plasma. It has been previously reported that membrane-bound SSAO is overexpressed in the cerebrovascular tissue of Alzheimer's disease (AD) patients. The aim of the present work is to study whether the circulating SSAO is also altered in this neurodegenerative disease. SSAO activity was determine! d in plasma of control cases (n = 23) and patients suffering sporadic Alzheimer dementia, distributed according to the Global Deterioration Scale (GDS): mild (n = 33), moderate (n = 14), moderate-severe (n = 15) and severe dementia (n = 19). Results show a clear increase of plasma SSAO activity ($p < 0.001$) in moderate-severe and severe AD patients, with patient age being an independent correlative factor. However, plasma SSAO activity was not altered in AD patients with mild or moderate dementia compared to controls. beta-Amyloid (Abeta) (40-42) immunoreactivity in plasma samples was also determined, and no correlation was observed between Abeta 40-42 levels and the severity of the dementia or the plasma SSAO activity. The results suggest that an increase in circulating SSAO activity could contribute to oxidative stress and vascular damage in advanced Alzheimer's disease (del Mar Hernandez et al., 2005).

Oxidative stress and chronic inflammation are connected to the increase in soluble semicarbazide-sensitive amine oxidase activity (Yu et al., 2002). In the study of Yu et al, semicarbazide-sensitive amine oxidase has been recognized to be a potential risk factor in vascular disorders associated with diabetic complications and to be related to mortality in patients suffering from heart disease. This enzyme, associated with the vascular system, catalyses the deamination of methylamine and aminoacetone, and also acts as an adhesion molecule related to leukocyte trafficking and inflammation. The deaminated products include the toxic aldehydes, formaldehyde and methylglyoxal, respectively, hydrogen peroxide and ammonia. In this study, the KKAy mouse, a strain possessing features closely resembling those of Type II (non-insulin-dependent) diabetes mellitus has been used to substantiate the hypothesis. Vascular lesions were induced via chronic feeding of a high cholesterol diet! . Both MDL-72974A, a selective mechanism-based semicarbazide-sensitive amine oxidase inhibitor and aminoguanidine effectively inhibited aorta semicarbazide-sensitive amine oxidase activity, and caused a substantial increase in urinary methylamine, and a decrease in formaldehyde and methylgloxal levels. Inhibition of semicarbazide-sensitive amine oxidase also reduced oxidative stress, as shown by a reduction of malondialdehyde excretion. Both MDL-72974A and aminoguanidine reduced albuminuria, proteinuria and the number of atherosclerotic lesions in animals fed with a cholesterol diet over a period of treatment for 16 weeks. Increased semicarbazide-sensitive amine oxidase-mediated deamination could be involved in the cascade of atherogenesis related to diabetic complications (Yu et al., 2002). In addition, a relationship between oxidative stress, inflammation and the activity of soluble semicarbazide-sensitive amine oxidase is noticed in diabetic conditions, as well as being !

a possible risk factor for Alzheimer's disease is the most frequent form of dementia.

Facts that inflammation has an important role in the development of Alzheimer's disease originate from some past epidemiological studies demonstrating decrease in the risk of the Alzheimer disease related with extended management of non-steroidal anti-inflammatory drugs (Stewart et al., 1997). Stewart et al, has shown in his study that in a longitudinal study of 1,686 participants in the Baltimore Longitudinal Study of Aging, it was examined whether the risk of Alzheimer's disease (AD) was reduced among reported users of aspirin or other nonsteroidal anti-inflammatory drugs (NSAIDs). In addition, it was examined use of acetaminophen, a pain-relief medication with little or no anti-inflammatory activity, to assess the specificity of the association between AD risk and self-reported medications. Information on use of medications was collected during each biennial examination between 1980 and 1995. The relative risk (RR) for AD decreased with increasing duration of NSAID! use. Among those with 2 or more years of reported NSAID use, the RR was 0.40 (95% confidence interval [CI]: 0.19-0.84) compared with 0.65 (95% CI: 0.33-1.29) for those with less than 2 years of NSAID use. The overall RR for AD among aspirin users was 0.74 (95% CI: 0.46-1.18), and no trend of decreasing risk of AD was observed with increasing duration of aspirin use. No association was found between AD risk and use of acetaminophen (RR = 1.35; 95% CI: 0.79-2.30), and there was no trend of decreasing risk with increasing duration of use. These findings are consistent with evidence from cross-sectional studies indicating protection against AD risk among NSAID users and with evidence suggesting that an inflammatory process characterizes one stage of the pathophysiology leading to AD (Stewart et al., 1997).

The development of reactive oxygen species directing to oxidative stress is as

well the result of deficient metabolic control in diabetes, coming from hyperglycemia (Mohanty et al., 2000). In the study made by Mohanty et al, diabetes mellitus is associated with increased reactive oxygen species (ROS) generation, oxidative injury and obesity. To elucidate the relationship between nutrition and ROS generation, it has been investigated the effect of glucose challenge on ROS generation by leucocytes, p47phox protein, a key protein in the enzyme NADPH oxidase and alpha-tocopherol levels. Blood samples were drawn from 14 normal subjects prior to, at 1, 2 and 3 h following ingestion of 75 g glucose. ROS generation by polymorphonuclear leucocytes (PMNL) and mononuclear cells (MNC) increased to a peak of 244 +/- 42% and 233 +/- 34% of the basal respectively at 2h. The levels of p47phox in MNC homogenates increased significantly at 2 h and 3 h after glucose intake. alpha-Tocopherol le! vels decreased significantly at 1 h, 2 h and 3 h. It was concluded that glucose intake stimulates ROS generation and p417phox of NADPH oxidase; increases oxidative load and causes a fall in alpha-tocopherol concentration (Mohanty et al., 2000).

Medical and scientific data specifies that oxidative stress imbalance and subsequent chronic oxidative stress are not only basic proceedings, but they too have a performing part in the development of Alzheimer’s disease (Pratico et al., 2004). In the study of Practico et al, Alzheimer's disease (AD) is a chronic neurodegenerative disorder that impairs cognition and behavior. Although the initiating molecular events are not known, increasing evidence suggests that oxidative stress could play a functional role in its pathogenesis. Lipoxygenase (LOX) enzymes by oxidizing polyunsaturated fatty acids synthesize hydroperoxyacids, which are potent pro-oxidant mediators. Because circumstantial evidence suggests that 12/15-LOX is a major source of oxidative stress, it

has been investigated the protein levels and activity of this enzyme in different brain regions of histopathologically confirmed AD and control cases. Using quantitative Western blot analysis it was demonstrated ! that in affected frontal and temporal regions of AD brains the amount of 12/15-LOX was higher compared with controls, whereas no difference between the two groups was detected in the cerebellum. This observation was confirmed by immunohistochemical studies. Levels of 12/15-hydroxyeicosatetraenoic acids, metabolic products of 12/15-LOX, were also markedly elevated in AD brains compared to controls. This increase directly correlated with brain lipid peroxidation, and inversely with vitamin E levels. Finally, genetic deletion of this enzyme in vitro resulted in a reduction of the cellular oxidative stress response after incubation with H2O2 or amyloid beta. These data show that the 12/15-LOX metabolic pathway is increased and correlates with an oxidative imbalance in the AD brain, implying that this enzyme might contribute to the pathogenesis of this neurodegenerative disorder (Pratico et al., 2004).

OXIDATIVE STRESS AND MITOCHONDRIA

Mitochondria are very important organelles that have some roles in the cell, which are amino acid biosynthesis, fatty acid oxidation, and steroid metabolism. Furthermore, mitochondria preserve the cellular energy reserves with ATP production by the electron transport of the respiratory chain. In addition, it is also recognized to be a very important source of superoxide radicals and other reactive oxygen species that are connected with oxidative stress (Jang et al., 2000). In the research of Jang et al, increased oxidative stress has been suggested to be involved in the pathogenesis and progression of

diabetic tissue damage. Several antioxidants have been described as beneficial for oxidative stress-associated diseases. Boldine ([s]-2,9-dihydroxy-1, 10-dimethoxyaporphine) is a major alkaloid found in the leaves and bark of boldo (Peumus boldus Molina), and has been shown to possess antioxidant activity and anti-inflammatory effects. From this point of view, the possi! ble anti-diabetic effect of boldine and its mechanism were evaluated. The experiments were performed on male rats divided into four groups: control, boldine (100 mg kg (-1), daily in drinking water), diabetic [single dose of 80 mg kg(-1)of streptozotocin (STZ), i.p.] and diabetic simultaneously fed with boldine for 8 weeks. Diabetic status was evaluated periodically with changes of plasma glucose levels and body weight in rats. The effect of boldine on the STZ-induced diabetic rats was examined with the formation of malondialdehydes and carbonyls and the activities of endogenous antioxidant enzymes (superoxide dismutase and glutathione peroxidase) in mitochondria of the pancreas, kidney and liver. The scavenging action of boldine on oxygen free radicals and the effect on mitochondrial free radical production were also investigated. The treatment of boldine attenuated the development of hyperglycemia and weight loss induced by STZ injection in rats. The levels of malondialde! hyde (MDA) and carbonyls in liver, kidney and pancreas mitochondria were significantly increased in STZ-treated rats and decreased after boldine administration. The activities of mitochondrial manganese superoxide dismutase (MnSOD) in the liver, pancreas and kidney were significantly elevated in STZ-treated rats. Boldine administration decreased STZ-induced elevation of MnSOD activity in kidney and pancreas mitochondria, but not in liver mitochondria. In the STZ-treated group, glutathione peroxidase activities decreased in liver mitochondria, and were

elevated in pancreas and kidney mitochondria. The boldine treatment restored the altered enzyme activities in the liver and pancreas, but not the kidney. Boldine attenuated STZ- and iron plus ascorbate-induced MDA and carbonyl formation and thiol oxidation in the pancreas homogenates. Boldine decomposed superoxide anions, hydrogen peroxides and hydroxyl radicals in a dose-dependent manner. The alkaloid significantly attenuated the production of superoxide anions, hydrogen peroxide and nitric oxid! e caused by liver mitochondria. The results indicate that boldine may exert an inhibitory effect on STZ-induced oxidative tissue damage and altered antioxidant enzyme activity by the decomposition of reactive oxygen species and inhibition of nitric oxide production and by the reduction of the peroxidation-induced product formation. Boldine may attenuate the development of STZ-induced diabetes in rats and interfere with the role of oxidative stress, one of the pathogeneses of diabetes mellitus (Jang et al., 2000).

Mitochondria Dysfunction and Diabetic Complications

Mitochondria dysfunction has been anticipated to be the intermediary between neurodegeneration in the central nervous system and peripheral nervous system and it has been talked about to be a serious transformer of diabetic complications in neurons by directing neurons to death, and endothelial cells (Schapira, 1998; Nishikawa et al., 2000). In the study of Nishikawa et al, diabetic hyperglycemia causes a variety of pathological changes in small vessels, arteries and peripheral nerves. Vascular endothelial cells are important targets of hyperglycemic damage, but the mechanisms underlying this damage are not fully understood. Three seemingly independent biochemical pathways are

involved in the pathogenesis: glucose-induced activation of protein kinase C isoforms; increased formation of glucose-derived advanced glycation end products; and increased glucose flux through the aldose reductase pathway. The relevance of each of these pathways is supported by animal studies in which! pathway-specific inhibitors prevent various hyperglycemia-induced abnormalities. Hyperglycemia increases the production of reactive oxygen species inside cultured bovine aortic endothelial cells. It was shown that an inhibitor of electron transport chain complex II prevents this increase in reactive oxygen species, by an uncoupler of oxidative phosphorylation, by uncoupling protein-1 and by manganese superoxide dismutase. Normalizing levels of mitochondrial reactive oxygen species with each of these agents prevents glucose-induced activation of protein kinase C, formation of advanced glycation end products, sorbitol accumulation and NFkappaB activation (Nishikawa et al., 2000). In the study of Schapira et al, mutations of mitochondrial DNA (mtDNA) are associated with a wide spectrum of disorders encompassing the myopathies, encephalopathies and cardiomyopathies, in addition to organ specific presentations such as diabetes mellitus and deafness. The pathogenesis of mtDNA mu! tations is not fully understood although it is assumed that their final common pathway involves impaired oxidative phosphorylation. The identification of a specific respiratory chain defect (complex I deficiency) in Parkinson's disease (PD) 10 years ago focused attention on the etiological and pathogenetic roles that mitochondria may play in neurodegenerative diseases. There is evidence now emerging that mtDNA abnormalities may determine the complex I defect in a proportion of PD patients and it may prove possible to use biochemical analysis of platelet and cybrid complex I function to identify those that lie within this group. Respiratory chain defects of a different pattern

have been identified in Huntington's disease (HD) (complex II/III deficiency) and Friedreich's ataxia (FA) complex I-III deficiency). In both these disorders, the mitochondrial abnormality is secondary to the primary nuclear mutation:CAG repeat in the huntingtin gene in HD, and GAA repeat in the frataxin gene in FA. Nevertheless, it appears that the mitochondrion may be t! he target of the biochemical defects that are the consequence of these mutations. There is a close and reciprocal relationship between respiratory chain dysfunction and free radical generation, and there is evidence for oxidative stress and damage in PD, HD and FA, which together with the mitochondrial defect may result in cell damage. Impaired oxidative phosphorylation and free radical generation may independently adversely affect the maintenance of mitochondrial transmembrane potential (Deltapsim). A fall in Deltapsim is an early event (preceding nuclear fragmentation) in the apoptotic pathway. It is possible therefore that mitochondrial dysfunction in the neurodegenerative disorders may result in a fall in the apoptotic threshold of neurons which, in some, may be sufficient to induce cell death whilst, in others, additional factors may be required. In any event, mitochondria present an important target for future strategies for 'neuroprotection' to prevent or retard neur! odegeneration (Schapira, 1998).

In the peripheral nerve of people with diabetes, mitochondrial ballooning and interference of inner cristae can be found, it is localized in Schwann cells and is hardly ever noticed in the axon (Kalichman et al., 1998). In the study of Kalichman et al, despite early descriptions of hypertrophic Schwann cells and onion-bulb formation in patients with diabetic neuropathy, clinical and experimental studies have emphasized axonal pathology. In recent years, the Schwann cell has been further implicated in diabetic

neuropathy because it is the primary intrafascicular location for the first enzyme of the polyol pathway, aldose reductase, which appears to have a role in modulating a variety of complications of diabetes, including diabetic neuropathy. To further explore the role of polyol pathway flux in the pathogenesis of Schwann cell injury, ultrastructural abnormalities of Schwann cells in human diabetic neuropathy (HDN) were compared with those in experimental galactose neuropat! hy (EGN), a well-characterized model of hyperglycemia without hypoinsulinemia. Similar to previous studies of EGN, reactive, degenerative and proliferative changes of Schwann cells were observed after 2, 4 and 24 months of galactose intoxication. Reactive changes included accumulation of lipid droplets, pi granules of Reich and glycogen granules, increased numbers of subplasmalemmal vesicles, cytoplasmic expansion, and capping. Degenerative changes included enlargement of mitochondria and effacement of cristae, and disintegration of both abaxonal and adaxonal cytosol and organelles. Both demyelination and onion-bulb formation were seen at all time points, although supernumerary Schwann cells and axonal degeneration were most numerous after 24 months of galactose feeding. In sural nerve biopsy samples from patients with diabetes and progressive worsening of neuropathy, ultrastructural abnormalities in Schwann cells encompassed the full range of reactive, degenerative and pro! liferative changes described in galactose-fed rats. The concordance of fine-structural observations in nerves from galactose-fed rats and these adult-onset diabetic patients emphasizes the role of flux through aldose reductase in the complex pathology of diabetic neuropathy and points to the utility of galactose intoxication in helping to understand this metabolic disorder (Kalichman et al., 1998).

Mitochondria changed by oxidative stress are believed to have a significant role

in cell death in either two ways, which are: through apoptosis or through necrosis (Tan et al., 1998). In the study of Tan et al, oxidative stress is implicated in a number of neurological disorders including stroke, Parkinson's disease, and Alzheimer's disease. To study the effects of oxidative stress on neuronal cells, it has been used an immortalized mouse hippocampal cell line (HT-22) that is particularly sensitive to glutamate. In these cells, glutamate competes for cysteine uptake, leading to a reduction in glutathione and, ultimately, cell death. As it has been reported that protein kinase C activation inhibits glutamate toxicity in these cells and is also associated with the inhibition of apoptosis in other cell types, it was asked if glutamate toxicity was via apoptosis. Morphologically, glutamate-treated cells underwent plasma membrane blebbing and cell shrinkage, but no DNA fragmenta! tion was observed. At the ultrastructural level, there was damage to mitochondria and other organelles although the nuclei remained intact. Protein and RNA synthesis inhibitors as well as certain protease inhibitors protected the cells from glutamate toxicity. Both the macromolecular synthesis inhibitors and the protease inhibitors had to be added relatively soon after the addition of glutamate, suggesting that protein synthesis and protease activation are early and distinct steps in the cell death pathway. Thus, the oxidative stress brought about by treatment with glutamate initiates a series of events that lead to a form of cell death distinct from either necrosis or apoptosis (Tan et al., 1998). Furthermore, mitochondria have the ability to reduce and to regulate the oxidative status of the cell. Antioxidants decrease age related oxidative stress and harms to mitochondria DNA (de la Asuncion et al., 1996). In the study made by de la Asuncion et al, mitochondria may be pr! imary targets of free radical damage associated with aging. It was found that mitochondrial glutathione is markedly oxidized with aging

in rats and mice. The oxidized to reduced glutathione ratio rises with aging in the liver, kidney, and brain. The magnitude of these changes is much higher than that previously found in whole cells of any species previously studied. In the liver, this ratio (expressing GSSG as a percent of GSH) changed from 0.77 +/- 0.19% (n=5) in young rats to 2.47 +/- 1.25% (n=5) in old ones, i.e., 320% of the controls. In the brain and kidney, values for old rats were, respectively, 600 and 540% higher than those of young rats. A marked oxidation of mitochondrial glutathione also occurred in mice. Aging also caused an increase in 8-oxo-7,8-dihydro-2'-deoxyguanosine levels in mtDNA in rats and mice. Oral antioxidant administration protected against both glutathione oxidation and mtDNA damage in rats and mice. Finally, it was found a direct relationship between mtDNA damage and mitochondrial glutathione oxidation. This occurs! both in rats (r=0.95) and in mice (r=0.98). This relationship, which has been observed for the first time in these studies, underscores the role of glutathione in the protection against free radical damage that occurs upon aging (de la Asuncion et al., 1996).

Mitochondria have also a significant role in the progression of diabetes type 2 that settles with the age in the capacity for oxidative phosphorylation, and as a result participates to its pathophysiology (Moreira et al., 2005). In the study of Moreira et al, using brain mitochondria isolated from 20-month-old diabetic Goto-Kakizaki rats, it was evaluated the efficacy of CoQ10 treatment against mitochondrial dysfunction induced by Abeta1-40. For that purpose, several mitochondrial parameters were evaluated: respiratory indexes (RCR and ADP/O ratio), transmembrane potential (DeltaPsim), repolarization lag phase, repolarization and ATP levels and the capacity of mitochondria to produce hydrogen peroxide. It has been observed that 4 microM Abeta1-40 induced a

significant decrease in the RCR and ATP content and a significant increase in hydrogen peroxide production. CoQ10 treatment attenuated the decrease in oxidative phosphorylation efficiency and avoided the increase in hydrog! en peroxide production induced by the neurotoxic peptide. These results indicate that CoQ10 treatment counteracts brain mitochondrial alterations induced by Abeta1-40 suggesting that CoQ10 therapy can help to avoid a drastic energy deficiency that characterizes diabetes and Alzheimer's disease pathophysiology (Moreira et al., 2005). Mitochondria are very necessary for neuronal functions because of the partial glycolytic space of these cells, which makes them reliant on aerobic oxidative phosphorylation intended for their active requirements. In addition, mitochondria play an essential role in the function of the cell and seem to represent one of the principal sources of reactive oxygen species (Kowaltowski and Vercesi, 1999). In the review written by Kowaltowski et al, Up to 2% of the oxygen consumed by the mitochondrial respiratory chain undergoes one electron reduction, typically by the semiquinone form of coenzyme Q, to generate the superoxide radical, and subsequently o! ther reactive oxygen species such as hydrogen peroxide and the hydroxyl radical. Under conditions in which mitochondrial generation of reactive oxygen species is increased (such as in the presence of Ca2+ ions or when the mitochondrial antioxidant defense mechanisms are compromised), these reactive oxygen species may lead to irreversible damage of mitochondrial DNA, membrane lipids and proteins, resulting in mitochondrial dysfunction and ultimately cell death (Kowaltowski and Vercesi, 1999).

Mitochondrial oxidative damage is assumed to implicate in the pathogenesis of a number of diseases especially in myocardial injury (Adlam et al., 2005). In the study of

Adlam et al, mitochondrial oxidative damage contributes to a wide range of pathologies, including cardiovascular disorders and neurodegenerative diseases. Therefore, protecting mitochondria from oxidative damage should be an effective therapeutic strategy. However, conventional antioxidants have limited efficacy due to the difficulty of delivering them to mitochondria in situ. To overcome this problem, it has been developed mitochondria-targeted antioxidants, typified by MitoQ, which comprises a lipophilic triphenylphosphonium (TPP) cation covalently attached to an ubiquinol antioxidant. Driven by the large mitochondrial membrane potential, the TPP cation concentrates MitoQ several hundred-fold within mitochondria, selectively preventing mitochondrial oxidative damage. To test whether MitoQ was active in vivo! , we chose a clinically relevant form of mitochondrial oxidative damage: cardiac ischemia-reperfusion injury. Feeding MitoQ to rats significantly decreased heart dysfunction, cell death, and mitochondrial damage after ischemia-reperfusion. This protection was due to the antioxidant activity of MitoQ within mitochondria, as an untargeted antioxidant was ineffective and accumulation of the TPP cation alone gave no protection. Therefore, targeting antioxidants to mitochondria in vivo is a promising new therapeutic strategy in the wide range of human diseases such as Parkinson's disease, diabetes, and Friedreich's ataxia where mitochondrial oxidative damage underlies the pathology (Adlam et al., 2005).

The first indication of mitochondrial dysfunction in diabetes was about 11 years ago, when the information was brought up that enlarged proportion of glycotic to oxidative enzyme activity in diabetes. The malfunctioning mitochondrial respiration has been credited to some genetic and acquired imperfections in oxidative respiration

(Bonawitz et al., 2006). In the study made by Bonawitz et al, mitochondrial dysfunction causes numerous human diseases and is widely believed to be involved in aging. However, mechanisms through which compromised mitochondrial gene expression elicits the reported variety of cellular defects remain unclear. The amino-terminal domain (ATD) of yeast mitochondrial RNA polymerase is required to couple transcription to translation during expression of mitochondrial DNA-encoded oxidative phosphorylation subunits. It was reported that several ATD mutants exhibit reduced chronological life span. The most severe of these (harborin! g the rpo41-R129D mutation) displays imbalanced mitochondrial translation, conditional inactivation of respiration, elevated production of reactive oxygen species (ROS), and increased oxidative stress. Reduction of ROS, via overexpression of superoxide dismutase (SOD1 or SOD2 product), not only greatly extends the life span of this mutant but also increases its ability to respire. Another ATD mutant with similarly reduced respiration (rpo41-D152A/D154A) accumulates only intermediate levels of ROS and has a less severe life span defect that is not rescued by SOD. Altogether, the results provide compelling evidence for the "vicious cycle" of mitochondrial ROS production and lead us to propose that the amount of ROS generated depends on the precise nature of the mitochondrial gene expression defect and initiates a downward spiral of oxidative stress only if a critical threshold is crossed (Bonawitz et al., 2006).

Mitochondrial Uncoupling Proteins

Uncoupling proteins are part of nuclear-encoded carriers that function in the same

way as proton carrier proteins in the internal membrane of mitochondria. Uncoupling proteins-induced protons leakiness initiates incomplete depolarization belonging to the mitochondrial transmembrane potential (Dulloo and Samec, 2001). In the review written by Dulloo et al, it was discussed about several new members of the mitochondrial carrier protein family that have been identified in a variety of tissues and organs. All apparently possess uncoupling properties in genetically-modified systems, with two of them (uncoupling protein (UCP) 2 and UCP3) being expressed in adipose tissues and skeletal muscles, which are generally recognized as important sites for variations in thermogenesis and/or in substrate oxidation. Considered as breakthrough discoveries, the cloning of these genes has generated considerable optimism for rapid advances in our molecular understanding ! of adaptive thermogenesis, and for the identification of new targets for pharmacological management of obesity and cachexia. The general conclusion is that UCP2 and UCP3 may have distinct primary functions, with UCP3 implicated in regulating the flux of lipid substrates across the mitochondria and UCP2 in the control of mitochondrial generation of reactive oxygen species. The distinct functions of these two UCP1 homologues have been incorporated in a conceptual model to illustrate how UCP2 and UCP3 may act in concert in the overall regulation of lipid oxidation concomitant to the prevention of lipid-induced oxidative damage (Dulloo and Samec, 2001). There are many types of uncoupling proteins and they are as follows: uncoupling protein 1, uncoupling protein 2, and uncoupling protein 3. All three of them have different functions and tissue distribution. Uncoupling protein 1 leads to thermogenesis, and the functions of the other two, which were uncoupling protein 2 and 3, are! not very clear but they are suspected of causing a weak uncoupling of respiration which leads to

mitochondrial membrane potential plus the buildup of oxygen radicals.

Uncoupling protein 1, in mice, shows an improved skeletal muscle glucose transport, in spite of decreased ATP substance and mitochondrial proteins (Han et al., 2004). In the study of Han et al, the aim was to address the potential role of lipotoxicity and mitochondrial function in insulin resistance. Mice with high-level expression of uncoupling protein-1 in skeletal muscle (UCP-H mice) were studied. Body weight, body length, and bone mineral density were decreased in UCP-H mice compared with wild-type littermates. Forelimb grip strength and muscle mass were strikingly decreased, whereas muscle triglyceride content was increased fivefold in UCP-H mice. Electron microscopy demonstrated lipid accumulation and large mitochondria with abnormal architecture in UCP-H skeletal muscle. ATP content and key mitochondrial proteins were decreased in UCP-H muscle. Despite mitochondrial dysfunction and increased intramyocellular fat, fasting serum glucose was 22% lower and insulin-stimula! ted glucose transport 80% higher in UCP-H animals. These beneficial effects on glucose metabolism were associated with increased AMP kinase and hexokinase activities, as well as elevated levels of GLUT4 and myocyte enhancer factor-2 proteins A and D in skeletal muscle. These results suggest that UCP-H mice have a mitochondrial myopathy due to depleted energy stores sufficient to compromise growth and impair muscle function. Enhanced skeletal muscle glucose transport in this setting suggests that excess intramyocellular lipid and mitochondrial dysfunction is not sufficient to cause insulin resistance in mice (Han et al., 2004).

In diabetes, which is high-fat-diet-induced, hepatic uncoupling protein 1 expression markedly enhanced insulin resistance (Ishigaki et al., 2005). In the study of

Ishigaki et al, it was examined whether dissipating excess energy in the liver is a possible therapeutic approach to high-fat diet-induced metabolic disorders, uncoupling protein-1 (UCP1) was expressed in murine liver using adenoviral vectors in mice with high-fat diet-induced diabetes and obesity, and in standard diet-fed lean mice. Once diabetes with obesity developed, hepatic UCP1 expression increased energy expenditure, decreased body weight, and reduced fat in the liver and adipose tissues, resulting in markedly improved insulin resistance and, thus, diabetes and dyslipidemia. Decreased expressions of enzymes for lipid synthesis and glucose production and activation of AMP-activated kinase in the liver seem to contribute to these improvements. Hepatic UCP1 expression also reversed high-fat diet-induced hyperp! hagia and hypothalamic leptin resistance, as well as insulin resistance in muscle. In contrast, intriguingly, in standard diet-fed lean mice, hepatic UCP1 expression did not significantly affect energy expenditure or hepatic ATP contents. Furthermore, no alterations in blood glucose levels, body weight, or adiposity were observed. These findings suggest that ectopic UCP1 in the liver dissipates surplus energy without affecting required energy and exerts minimal metabolic effects in lean mice. Thus, enhanced UCP expression in the liver is a new potential therapeutic target for the metabolic syndrome (Ishigaki et al., 2005).

Several laboratories have shown that uncoupling protein 2 expression is associated with the level of reactive oxygen species generation by respiring mitochondria (Negre-Salvayre et al., 1997). In the study of Negre-Salvayre et al, according to the state of mitochondrial respiration, the respiratory chain generates superoxide anions converted into hydrogen peroxide. Two uncoupling proteins (UCP) able to modulate the coupling between the respiratory chain and ATP synthesis are now identified and could be

involved in mitochondrial H2O2 generation. UCP1 is specific to brown adipose tissue (BAT) whereas UCP2 is expressed in numerous tissues, particularly in monocytes/macrophages. Preincubation of BAT mitochondrial fractions with GDP, an inhibitor of UCP1, induced a rise in mitochondrial membrane potential (assessed by rhodamine 123 uptake) and H2O2 production. An uncoupling agent reversed this effect. Liver mitochondria exhibited a similar phenotype. GDP was also able to raise me! mbrane potential and H2O2 production of the mitochondria from nonparenchymal cells expressing UCP2, but was completely ineffective on mitochondria from hepatocytes deprived of UCP2. The GDP effect was also observed with mitochondrial fractions of the spleen or thymus, which highly expressed UCP2. Altogether, these results strongly suggest that UCP2 is sensitive to GDP and that the UCPs, particularly UCP2, are able to modulate H2O2 mitochondrial generation. This supports a role for UCP2 in cellular (patho-) physiological processes involving free radicals generated by mitochondria, such as oxidative damage, inflammation, or apoptosis (Negre-Salvayre et al., 1997).

In diabetes, overexpression of the uncoupling of proteins in cultured neurons stops glucose-induced programmed cells death by avoiding mitochondrial hyperpolarization and the development of reactive oxygen species (Vincent et al., 2004). In the study of Vincent et al, it was studied the central role of mitochondria in most pathways leading to programmed cell death (PCD) has focused the investigations into the mechanisms of glucose-induced neuronal degeneration. It has been postulated that hyperglycemic neuronal injury results from mitochondria membrane hyperpolarization and reactive oxygen species formation. The present study not only provides further evidence to support our model of glucose-induced PCD but also demonstrates a potent

ability for uncoupling proteins (UCPs) to prevent this process. Dorsal root ganglion (DRG) neurons were screened for UCP expression by Western blotting and immunocytochemistry. The abilities of individual UCPs to prevent hyperglycemic PCD were ! assessed by adenovirus-mediated overexpression of UCP1 and UCP3. Interestingly, UCP3 is expressed not only in muscle, but also in DRG neurons under control conditions. UCP3 expression is rapidly downregulated by hyperglycemia in diabetic rats and by high glucose in cultured neurons. Overexpression of UCPs prevents glucose-induced transient mitochondrial membrane hyperpolarization, reactive oxygen species formation, and induction of PCD. The loss of UCP3 in DRG neurons may represent a significant contributing factor in glucose-induced injury. Furthermore, the ability to prevent UCP3 downregulation or to reproduce the uncoupling response in DRG neurons constitutes promising novel approaches to avert diabetic complications such as neuropathy (Vincent et al., 2004).

Beta cells were weak to oxidative stress and they were initiated to fight H2O2 toxicity through the generation of uncoupling protein 2 (Li et al., 2001). In the study of Li et al, the role of uncoupling protein-2 (UCP-2) in beta-cells is presently unclear. It has been tested the notion that UCP-2 participates in beta-cell defense against oxidants. Expression of the UCP-2 gene in clonal beta cells (INS-1) was decreased by 45% after 48 h of culture with vitamin E and selenite. When INS-1 cells were exposed to 200 microM H (2)O(2) for 5 min, the cell viability (MTT assay) decreased to 85 +/- 1, 61 +/- 1, 40 +/- 2, and 39 +/- 2% of control when measured respectively 30 min, 2 h, 6 h, and 16 h after H(2)O(2) exposure. At corresponding time points UCP-2 mRNA levels were 1.01 +/- 0.09, 1.53 +/- 0.15 ($P < 0.05$), 1.44 +/- 0.18 ($P = 0.06$), and 1.12 +/- 0.09 fold of control,

i.e., transiently increased. What was tested next was whether overexpression of UCP-2 could enhance resistance ! of beta-cells toward H(2)O(2) toxicity. A cotransfection method using EGFP as a suitable marker and a human cDNA UCP-2 construct was used for transient overexpression of UCP-2. Transfected cells expressed the gene about 30-fold more than normal cells. After exposure to H(2)O(2) (200 micrometer, 5 min), the survival of UCP-2 overexpressing cells was measured 30-45 min later by flow cytometry. Survival was 13 +/- 0.05% higher than control (EGFP only) cells, $P < 0.004$ for difference. The results indicate that oxidative stress induces UCP-2 expression in beta cells, and that UCP-2 serves a role in beta-cell defense against oxidative stress (Li et al., 2001). Furthermore, muscle cells over-expressing uncoupling protein 3 were revealed a significant decrease in mitochondrial reactive oxygen species manufacturing, even though there were no changes in glucose oxidation and mitochondrial membrane potential (MacLellan et al., 2005). In the study of MacLellan et al, Decreased uncoupli! ng protein (UCP)3 is associated with insulin resistance in muscle of pre-diabetic and diabetic individuals, but the function of UCP3 remains unclear. The goal was to elucidate mechanisms underlying the negative correlation between UCP3 and insulin resistance in muscle. It was determined effects of physiologic UCP3 overexpression on glucose and fatty acid oxidation and on mitochondrial uncoupling and reactive oxygen species (ROS) production in L6 muscle cells. An adenoviral construct caused a 2.2- to 2.5-fold increase in UCP3 protein. Palmitate oxidation was increased in muscle cells incubated under normoglycemic or hyperglycemic conditions, whereas adenoviral green fluorescent protein infection or chronic low doses of the uncoupler dinitrophenol had no effect. Increased UCP3 did not affect glucose oxidation, whereas dinitrophenol and insulin

treatments caused increases. Basal oxygen consumption, assessed in situ using self-referencing microelectrodes, was not significantly affected, whereas dinitrophenol caused increases. Mitochondrial membran! e potential was decreased by dinitrophenol but was not affected by increased UCP3 expression. Finally, mitochondrial ROS production decreased significantly with increased UCP3 expression. Results are consistent with UCP3 functioning to facilitate fatty acid oxidation and minimize ROS production. As impaired fatty acid metabolism and ROS handling are important precursors in muscular insulin resistance, UCP3 is an important therapeutic target in type 2 diabetes (MacLellan et al., 2005).

Uncoupling proteins reduce the mitochondrial reactive species initiation, and besides that, they play a significant role in finding the relationship in regulating fatty acids and glucose oxidation (Boss et al., 2000). In the review written by Boss et al, it is discussed that mitochondria use energy derived from fuel combustion to create a proton electrochemical gradient across the mitochondrial inner membrane. This intermediate form of energy is then used by ATP synthase to synthesize ATP. Uncoupling protein-1 (UCP1) is a brown fat-specific mitochondrial inner membrane protein with proton transport activity. UCP1 catalyzes a highly regulated proton leak, converting energy stored within the mitochondrial proton electrochemical potential gradient to heat. This uncouples fuel oxidation from conversion of ADP to ATP. In rodents, UCP1 activity and brown fat contribute importantly to whole-body energy expenditure. Recently, two additional mitochondrial carriers with high similari! ty to UCP1 were molecularly cloned. In contrast to UCP1, UCP2 is expressed widely, and UCP3 is expressed preferentially in skeletal muscle. Biochemical studies indicate that UCP2 and UCP3, like UCP1, have

uncoupling activity. While UCP1 is known to play an important role in regulating heat production during cold exposure, the biological functions of UCP2 and UCP3 are unknown. Possible functions include 1) control of adaptive thermogenesis in response to cold exposure and diet, 2) control of reactive oxygen species production by mitochondria, 3) regulation of ATP synthesis, and 4) regulation of fatty acid oxidation (Boss et al., 2000).

Uncoupling protein 3 is planned to take out the fatty acids from mitochondria and allow high levels of fatty acid oxidation as being the fatty acid over-supply. Uncoupling protein 3 can also be as a shield for mitochondria because of the fatty acids that can damage it (Carley and Severson, 2005). In the review written by Carley et al, the metabolic phenotype of hearts has been investigated using rodent models of type 2 diabetes which exhibit obesity and insulin resistance: db/db and ob/ob mice, and Zucker fatty and ZDF rats. In general, cardiac fatty acid (FA) utilization is enhanced in type 2 diabetic hearts, with increased rates of FA oxidation (db/db, ob/ob and ZDF models) and increased FA esterification into cellular triacylglycerols (db/db hearts). Hearts from db/db and ob/ob mice and ZDF rat hearts all have elevated levels of myocardial triacylglycerols, consistent with enhanced FA utilization. A number of mechanisms may be responsible for enhanced FA utilization in t! ype 2 diabetic hearts: (i) increased FA uptake into cardiac myocytes and into mitochondria; (ii) altered mitochondrial function, with up-regulation of uncoupling proteins; and (iii) stimulation of peroxisome proliferator-activated receptor-alpha. Enhanced cardiac FA utilization in rodent type 2 diabetic models is associated with reduced cardiac contractile function, perhaps as a consequence of lipotoxicity and/or reduced cardiac efficiency. Similar results have been

obtained with human type 2 diabetic hearts, suggesting that pharmacological interventions that can reduce cardiac FA utilization may have beneficial effects on contractile function (Carley and Severson, 2005).

In the same way as impaired fatty acid metabolism and reactive oxygen species management are important in muscular insulin resistance, uncoupling proteins seem to be probable therapeutic targets in type 2 diabetes (Langin, 2003). In the review of Langin et al, uncoupling proteins (UCP) are carriers expressed in the mitochondrial inner membrane that uncouple oxygen consumption by the respiratory chain from ATP synthesis. UCP2 is a member of the multigenic UCP family that is expressed in a wide range of tissues and organs. Possible functions of UCP2 include control of ATP synthesis, regulation of fatty acid metabolism and control of reactive oxygen species production. UCP2 expression in tissues involved in lipid and energy metabolism and mapping of the gene to a region linked to obesity and hyperinsulinemia prompted studies on the involvement of UCP2 in metabolic disorders, and especially in type 2 diabetes. In human adipose tissue and skeletal muscle, UCP2 expression is incre! ased during fasting. The carrier was shown to be under the control of fatty acids and thyroid hormones in vivo. An upregulation has been observed in the liver during high-fat feeding and obesity. However, data in UCP2 gene knockout mice do not support a role for UCP2 in steatohepatitis. The most compelling metabolic role of UCP2 comes from studies in pancreatic beta cells. Overexpression in isolated pancreatic islets results in decreased ATP content and blunted glucose-stimulated insulin secretion. UCP2-deficient mice show an increased ATP level and an enhanced insulin secretion. Lack of UCP2 dramatically improves insulin secretion and decreases hyperglycemia in leptin-deficient mice. The role

of UCP2 in the control of insulin secretion constitutes, to date, the most pertinent path to investigate in a therapeutic perspective (Langin, 2003). In addition, uncoupling protein 3 is related to insulin resistance in the muscles of diabetics and prediabetics, while the uncoupling p! rotein 3 functions have no clear explanation (Schrauwen et al., 2001). In the study of Schrauwen et al, a role for uncoupling protein-3 (UCP3) in carbohydrate metabolism and in type 2 diabetes has been suggested. Mice overexpressing UCP3 in skeletal muscle showed reduced fasting plasma glucose levels, improved glucose tolerance after an oral glucose load, and reduced fasting plasma insulin levels. However, data regarding the expression of UCP3 in patients with type 2 diabetes is inconsistent, and so far, there have been no reports of UCP3 protein content. It was compared, for the first time, the protein levels of UCP3 in vastus lateralis muscle in 14 male type 2 diabetic patients (age 49.8 +/- 2.1 years; BMI 27.2 +/- 1.2 kg/m(2); mean +/- SE) with 16 male control subjects (age 48.0 +/- 1.9 years; BMI 23.4 +/- 0.6 kg/m(2)). It was found that UCP3 protein levels were twice as low in patients with type 2 diabetes compared with control subjects (117 +/- 16 vs. 58 +/- 12 AU; P = 0.007). There was no correlation between UCP3 content and BMI. In conc! lusion, UCP3 content is lower in type 2 diabetic patients compared with healthy control subjects. These results are consistent with a role for UCP3 in glucose homeostasis and suggest a role for UCP3 in type 2 diabetes (Schrauwen et al., 2001).

Mitochondrial Function in Diabetes

Mitochondria have a very significant function in managing the life and the death

of a cell (Inoue et al., 2003). In the review written by Inoue et al, although oxygen is required for the energy metabolism in aerobic organisms, it generates reactive oxygen and nitrogen species that impair a wide variety of biological molecules, including lipids, proteins, and DNA, thereby causing various diseases. Because mitochondria are the major site of free radical generation, they are highly enriched with enzymes, such as Mn-type superoxide dismutase in matrix, and antioxidants including GSH on both sides of inner membranes, thus minimizing oxidative stress in and around this organelle. A cross talk of nitric oxide and oxygen radicals regulates the circulation, energy metabolism, reproduction, and remodeling of cells during embryonic development, and functions as a major defense system against pathogens. Cu/Zn-type superoxide dismutase, which has been postulated for a long time to! be a cytosolic enzyme, also localizes bound to inner membranes of mitochondria, thereby minimizing oxidative stress in and around this organelle, while mitochondrial association decreases markedly with the variant types of the enzyme found in patients with familial amyotrophic lateral sclerosis. It was also reported that a cross talk of nitric oxide, superoxide, and molecular oxygen cooperatively regulates the fates of pathogens and their hosts and that oxidative stress in and around mitochondria also determines cell death in the development of animals and tissue injury caused by anticancer agents by some carnitine-inhibitable (Inoue et al., 2003).

Being recognized as a mediator of the life and the death of a cell, mitochondria have drawn alertness that is needed to exploit antioxidants and new cytoprotective agents (Anders et al., 2006). In the study of Anders et al, the identification of the mitochondrion as the gatekeeper of the life and death of a cell and the appreciation of the role of mitochondrial dysfunction in a range of clinical disease processes have made the

mitochondrion a target for drug delivery. Accordingly, strategies are being developed for the targeted delivery of antioxidants to mitochondria. Recent studies show that triphenylphosphonium-based antioxidants and amino acid- and peptide-based antioxidants protect mitochondria against oxidative insult. Future studies will undoubtedly exploit the unique biophysical and biochemical properties of mitochondria, including mitochondrial activation of prodrugs, for the targeted delivery of cytoprotective agents (Anders et al., 2006). The destruction of mitoc! hondrial function is essentially connected with diabetes. The examination that lowered the rates of ATP synthesis in themes among a family history of diabetes arose previous to the beginning of impaired glucose tolerance. Petersen et al, specifies the importance of mitochondrial dysfunction in diabetes development (Petersen et al., 2004). In Petersen et al, Insulin resistance appears to be the best predictor of the development of diabetes in the children of patients with type 2 diabetes, but the mechanism responsible is unknown. It was performed hyperinsulinemic-euglycemic clamp studies in combination with infusions of [6,6-(2)H(2)]glucose in healthy, young, lean, insulin-resistant offspring of patients with type 2 diabetes and insulin-sensitive control subjects matched for age, height, weight, and physical activity to assess the sensitivity of liver and muscle to insulin. Proton ((1)H) magnetic resonance spectroscopy studies were performed to measure intramyocellular lipid! and intrahepatic triglyceride content. Rates of whole-body and subcutaneous fat lipolysis were assessed by measuring the rates of [(2)H(5)]glycerol turnover in combination with microdialysis measurements of glycerol release from subcutaneous fat. It was performed (31)P magnetic resonance spectroscopy studies to assess the rates of mitochondrial oxidative-phosphorylation activity in muscle. The insulin-stimulated rate

of glucose uptake by muscle was approximately 60 percent lower in the insulin-resistant subjects than in the insulin-sensitive control subjects (P<0.001) and was associated with an increase of approximately 80 percent in the intramyocellular lipid content (P=0.005). This increase in intramyocellular lipid content was most likely attributable to mitochondrial dysfunction, as reflected by a reduction of approximately 30 percent in mitochondrial phosphorylation (P=0.01 for the comparison with controls), since there were no significant differences in systemic or localized rates of lipolysis or plasma concentrations of tumor necrosis ! factor alpha, interleukin-6, resistin, or adiponectin. These data support the hypothesis that insulin resistance in the skeletal muscle of insulin-resistant offspring of patients with type 2 diabetes is associated with deregulation of intramyocellular fatty acid metabolism, possibly because of an inherited defect in mitochondrial oxidative phosphorylation (Petersen et al., 2004).

Metabolic adjustments in liver mitochondria were estimated in both models, which were the Goto-Kakizaki (GK) rats and streptozotocin (STZ) treated rats, and they were both different representing differential adjustment mechanism to offset high glucose levels characteristic of the disease (Ferreira et al., 1999). In the study of Ferreira et al, Type 2 diabetes mellitus is one of the most common chronic metabolic diseases in man. Due to long-term complications of the disease, severely decreasing the quality of life of diabetic patients, early interventions to obviate the risk of complications are of major importance. Therefore, diabetic animal models are of major importance in research for interventional treatment of type 2 diabetes. In this work it was investigated the possible alterations in mitochondrial energetic metabolism of Goto-Kakizaki (GK) rats during the progression of the disease, since glucose metabolism is closely related to intracellular

ATP content. For that re! ason, respiratory indexes (state 4, state 3, RCR and ADP/O) were evaluated either in the presence of NAD- or FAD-linked substrates (glutamate + malate and succinate, respectively) in mitochondrial preparations of GK and control rats with 8, 12, 26 and 52 weeks of age. Until the age of 1 year (52 weeks) it was found no impairment of mitochondrial respiratory indexes both in the presence of glutamate + malate and succinate. In conclusion, this study indicates that GK rat is a good model for studying the initial events of diabetes, since it presents no impairment of liver mitochondrial functions during the first year of life, contrasting clearly with pharmacological induced diabetes (Ferreira et al., 1999).

Titration with oliglomycin shows that diabetic GK liver mitochondria necessitate extra oligomycin pulses to entirely eliminate phosphorylation, comparative to manage mitochondria and this indicate that liver mitochondria of diabetic GK rats are supplied with additional catalytic units qualified to manage mitochondria of regular rats (Palmeira et al., 1999). In Palmeira et al, study, liver mitochondrial bioenergetics of Goto-Kakizaki (GK) rats (a model of non-insulin dependent diabetes mellitus) reveals a Delta Psi upon energization with succinate significantly increased relatively to control animals. The repolarization rate following ADP phosphorylation is also significantly increased in GK mitochondria in parallel with increased ATPase activity. The increase in the repolarization rate and ATPase activity is presumably related to an improved efficiency of F(0)F(1)-ATPase, either from a better phosphorylative energy coupling or as a consequence of an enlarged number of cataly! tic units. Titrations with oligomycin indicate that diabetic GK liver mitochondria require excess oligomycin pulses to completely abolish phosphorylation, relative to control mitochondria. Therefore,

accepting that the number of operational ATP synthase units is inversely proportional to the amount of added oligomycin, it is concluded that liver mitochondria of diabetic GK rats are provided with extra catalytic units relative to control mitochondria of normal rats. Other tissues (kidney, brain and skeletal muscle) were evaluated for the same bioenergetic parameters, confirming that this feature is exclusive to liver from diabetic GK rats (Palmeira et al., 1999).

Both types of diabetes had an unpleasant effect on cardiac mitochondrial bioenergetics (Oliveira et al., 2004). In the study of Oliveira et al, the objective of this work was to test if injections of Vitamin E and Coenzyme Q10, alone and in combination, were able to modify mitochondrial performance in the hearts of GK rats. Several aspects of mitochondrial function were measured, such as the respiratory control ratio and the electric potential, as well as the mitochondrial accumulation of Vitamin E and Coenzymes Q9 and Q10. It was observed that only Vitamin E appeared to have a positive impact on the mitochondrial phosphorylation efficiency and on mitochondrial performance, namely on the ability to generate the electric transmembrane potential in the presence of supra-physiological calcium concentrations. Vitamin E administration also increased the mitochondrial concentration of Coenzyme Q10. None of the treatments was able to reverse the diabetic phenotype in GK rats. It w! as conclude that in this model of mild hyperglycemia, administration of antioxidants may have a marginal positive impact on mitochondrial function (Oliveira et al., 2004). Lowered mitochondrial calcium uptake was noticed in STZ rats, were treated, in heart mitochondria, and it was associated with improved receptiveness to mitochondrial permeability transition initiation rather than destruction to the calcium uptake machinery (Oliveira et al., 2003). In the study of

Oliveira et al, cardiac dysfunction is associated with diabetes. It was previously shown that heart mitochondria from diabetic rats have a reduced calcium accumulation capacity. The objective of this work was to determine whether the reduction in calcium accumulation by cardiac mitochondria from diabetic rats is related to an enhanced susceptibility to induction of the mitochondrial permeability transition. Streptozotocin-induced diabetic rats were used as a model to study the alterations caused by diabetes in th! e permeability transition, 21 days after streptozotocin administration. Heart mitochondria were isolated to evaluate respiratory parameters and susceptibility to the calcium-dependent permeability transition. The results show that streptozotocin diabetes facilitates the mitochondrial permeability transition in cardiac mitochondria, resulting in decreased mitochondrial calcium accumulation. It was also observed that heart mitochondria from diabetic rats had depressed oxygen consumption during the phosphorylative state (Oliveira et al., 2003). An interesting fact was that the heart mitochondria from GK rats were less vulnerable to the initiation of mitochondrial permeability transition, illustrating a larger amount of calcium buildup previous to the complete failure of mitochondrial impermeability (Oliveira et al., 2001). In the study of Oliveira et al, type 2 diabetes (or non-insulin dependent diabetes mellitus, NIDDM) is a common metabolic disease in man. The Goto-Kakizaki (GK) rat has been designed as a NIDDM model. Previous studies with this! strain have shown differences at the mitochondrial level. The mitochondrial permeability transition (MPT) is a widely studied phenomenon but yet poorly understood, that leads to mitochondrial dysfunction and cell death. The aim of this study was to compare the differences in susceptibility of induction of the MPT with calcium phosphate in GK and Wistar rats. The results show that heart

mitochondria from GK rats are less susceptible to the induction of MPT, and show a larger calcium accumulation before the overall loss of mitochondrial impermeability (Oliveira et al., 2001).

The theory in mitochondria of aging assumes that the increased manufacturing of reactive oxygen species, mitochondrial DNA damage accumulation, and progressive respiratory chain dysfunction are procedures by which mitochondria is part of the cause of the aging process (Savitha et al., 2005). In the study of Savitha et al, oxidative damage is hypothesized to accumulate throughout the lifetime of an organism, eventually giving rise to aging. The mitochondria may be the primary cellular source and target of endogenous ROS as they are produced as a normal byproduct of the electron transport system. Male albino Wistar rats were used in this study. The animals were divided into 6 groups, each group consisting of 6 animals. Groups I, III, and V were young, middle-aged and aged control rats and Groups II, IV, and VI were treated with carnitine (300 mg/kg bw) and dl-alpha-lipoic acid (150 mg/kg bw), respectively. After the treatment period, the animals were sacrificed and the heart a! nd skeletal muscle were removed for analysis. There was a significant reduction in the levels of antioxidants in both middle-aged and aged rats whereas the thiobarbituric acid reactive substances were found to increase. Co-supplementation of carnitine and lipoic acid improved the antioxidant status and brought down the levels of TBARS. Co-supplementation of lipoic acid with carnitine has a beneficial effect in reversing the age-related abnormalities seen in aging. This effect was associated with the decrease in free radical production and rise in antioxidant levels by carnitine and lipoic acid, thereby lowering oxidative stress (Savitha et al., 2005).

Cells that do not have enough mitochondrial DNA also loose their capability to

develop the improvement of insulin emission by glucose stimulation (Santos et al., 2006). In the study of Santos et al, it was previously shown that the protein subunit of telomerase, hTERT, has a bonafide N-terminal mitochondrial targeting sequence, and that ectopic hTERT expression in human cells correlated with increase in mtDNA damage after hydrogen peroxide treatment. In this study, it is shown, using a loxP hTERT construct, that this increase in mtDNA damage following hydrogen peroxide exposure is dependent on the presence of hTERT itself. Further experiments using a dominant negative hTERT mutant shows that telomerase must be catalytically active to mediate the increase in mtDNA damage. Etoposide, but not methylmethanesulfate, also promotes mtDNA lesions in cells expressing active hTERT, indicating genotoxic specificity in this response. Fibroblasts expressing hTERT not only show an approxi! mately 2-fold increase in mtDNA damage after oxidative stress but also suffer a 10-30-fold increase in apoptotic cell death as assayed by Annexin-V staining, caspase-3 activation and PARP cleavage. Mutations to the N-terminal mitochondrial leader sequence cause a complete loss of mitochondrial targeting without affecting catalytic activity. Cells carrying this mutated hTERT not only have significantly reduced levels of mtDNA damage following hydrogen peroxide treatment, but strikingly also do not shown any loss of viability or cell growth. Thus, localization of hTERT to the mitochondria renders cells more susceptible to oxidative stress-induced mtDNA damage and subsequent cell death, whereas nuclear-targeted hTERT, in the absence of mitochondrial localization, is associated with diminished mtDNA damage, increased cell survival and protection against cellular senescence (Santos et al., 2006).

OXIDATIVE STRESS IN INSULIN RESISTANCE AND DIABETES

Insulin resistance takes place when the cells no longer respond well to insulin. A growing number of clinical and experimental studies have indicated a hyperglycemia yield in the generation of reactive oxygen species, in the end leading to increased oxidative stress in a variety of tissues. The cause of oxidative stress in diabetes has been, and continues to be, the object of considerable clinical investigation. It has been well established that oxidative stress in the cells of peripheral nerves lead to diabetic complications like neuropathy; plus the oxidative stress began long before the neuropathic symptoms of pain, burning, and numbness appear. More considerable is how oxidative stress has an effect on blood sugar concentration. It has been made known that oxidative stress has the ability to lower the insulin sensitivity and injure the insulin-producing cells within the pancreas. Adipose tissue and muscle are the most significant tissues participating in the! development of insulin resistance. Oxidative stress modifies the signaling pathway within a cell installing insulin resistance (Evans et al., 2003). In the review of Evans et al, in both type 1 and type 2 diabetes, diabetic complications in target organs arise from chronic elevations of glucose. The pathogenic effect of high glucose, possibly in concert with fatty acids, is mediated to a significant extent via increased production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) and subsequent oxidative stress. ROS and RNS directly oxidize and damage DNA, proteins, and lipids. In addition to their ability to directly inflict damage on macromolecules, ROS and RNS indirectly induce damage to tissues by activating a number of cellular stress-sensitive pathways. These pathways include nuclear factor-kappaB, p38 mitogen-

activated protein kinase, NH(2)-terminal Jun kinases/stress-activated protein kinases, hexosamines, and others. In addition, there is eviden! ce that in type 2 diabetes, the activation of these same pathways by elevations in glucose and free fatty acid (FFA) levels leads to both insulin resistance and impaired insulin secretion. Therefore, it is proposed that the hyperglycemia-induced, and possibly FFA-induced, activation of stress pathways plays a key role in the development of not only the late complications in type 1 and type 2 diabetes, but also the insulin resistance and impaired insulin secretion seen in type 2 diabetes (Evans et al., 2003).

Oxidative Stress in Insulin Resistance Caused by Agents

The following agents cause insulin resistance: obesity, hormone excess, pregnancy and a life style that is not physically active. Oxidative stress participates in the progression of insulin resistance (Evans et al., 2002). In the review of Evans et al, in both type 1 and type 2 diabetes, the late diabetic complications in nerve, vascular endothelium, and kidney arise from chronic elevations of glucose and possibly other metabolites including free fatty acids (FFA). Recent evidence suggests that common stress-activated signaling pathways such as nuclear factor-kappaB, p38 MAPK, and NH2-terminal Jun kinases/stress-activated protein kinases underlie the development of these late diabetic complications. In addition, in type 2 diabetes, there is evidence that the activation of these same stress pathways by glucose and possibly FFA leads to both insulin resistance and impaired insulin secretion. Thus, we propose a unifying hypothesis whereby hyperglycemia and FFA-induced activatio! n of the nuclear factor-kappaB, p38 MAPK,

and NH2-terminal Jun kinases/stress-activated protein kinases stress pathways, along with the activation of the advanced glycosylation end-products/receptor for advanced glycosylation end-products, protein kinase C, and sorbitol stress pathways, plays a key role in causing late complications in type 1 and type 2 diabetes, along with insulin resistance and impaired insulin secretion in type 2 diabetes. Studies with antioxidants such as vitamin E, alpha-lipoic acid, and N-acetylcysteine suggest that new strategies may become available to treat these conditions (Evans et al., 2002).

Insulin Resistance in Antioxidants Treatment

Antioxidants treatment kept hyperglycemia-induced insulin resistance in vivo (Haber et al., 2003). In Haber et al, study, exposure to high concentrations of glucose and insulin results in insulin resistance of metabolic target tissues, a characteristic feature of type 2 diabetes. High glucose has also been associated with oxidative stress, and increased levels of reactive oxygen species have been proposed to cause insulin resistance. To determine whether oxidative stress contributes to insulin resistance induced by hyperglycemia in vivo, nondiabetic rats were infused with glucose for 6 h to maintain a circulating glucose concentration of 15 mM with and without coinfusion of the antioxidant N-acetylcysteine (NAC), followed by a 2-h hyperinsulinemic-euglycemic clamp. High glucose (HG) induced a significant decrease in insulin-stimulated glucose uptake [tracer-determined disappearance rate (Rd), control 41.2 +/- 1.7 vs. HG 32.4 +/- 1.9 mg. kg-1. min-1, $P < 0.05$], which was pre! vented by NAC (HG + NAC 45.9 +/- 3.5 mg. kg-1. min-1). Similar results were obtained with the antioxidant taurine. Neither

NAC nor taurine alone altered Rd. HG caused a significant (5-fold) increase in soleus muscle protein carbonyl content, a marker of oxidative stress that was blocked by NAC, as well as elevated levels of malondialdehyde and 4-hydroxynonenal, markers of lipid peroxidation, which were reduced by taurine. In contrast to findings after long-term hyperglycemia, there was no membrane translocation of novel isoforms of protein kinase C in skeletal muscle after 6 h. These data support the concept that oxidative stress contributes to the pathogenesis of hyperglycemia-induced insulin resistance (Haber et al., 2003).

Oxidative stress has an important contribution, in vivo, to adjust the first stage of insulin emission for the reason that the latter has the ability to be restored through antioxidants {Paolisso, 1996 #34}. In Paolisso et al, study, in aged healthy (n = 10) and non-insulin-dependent (type II) diabetic (n = 10) subjects matched for age [67.3 +/- 0.5 vs. 68.0 +/- 0.4 yr, P = not significant (NS)], body mass index (25.7 +/- 0.7 vs. 26.0 +/- 0.2 kg/m2, P = NS), gender ratio [6 males (M)/4 females (F) vs. 5 M/5 F], and mean arterial blood pressure (104 +/- 6 vs. 105 +/- 9 mmHg, P = NS), it was determined the changes in insulin secretion and action after vitamin C infusion and the relative increase in plasma vitamin C levels. At the highest vitamin C infusion rate (0.9 mmol/min) the increase in plasma vitamin C levels did not affect B cell response to glucose, but it improved Conard's K values and whole body glucose disposal in healthy subjects and in diabetic patients. In both! groups of subject vitamin C-mediated increase in insulin action was mainly due to an improvement in nonoxidative glucose metabolism. After fasting, plasma vitamin C levels correlated with basal whole body glucose disposal ($r = -0.44$, $P < 0.05$; $n = 20$). After vitamin C infusion, percent change in plasma vitamin C

level correlated with the percent decline in membrane microviscosity ($r = 0.53$, $P < 0.01$; $n = 20$) and increase in whole body glucose disposal ($r = 0.63$, $P < 0.003$; $n = 20$). In conclusion, plasma vitamin C levels seem to play a role in the modulation of insulin action in aged healthy and diabetic subjects (Paolisso et al., 1994).

Type 1 and type 2 diabetes and cardiovascular disease have something in common which is that they share an ecological history. One thing they have in common is insulin resistance. Insulin resistance is the most important cause in the development of type 2 diabetes in older patients, related with reduced mitochondrial oxidative phosphorylation action (Lee et al., 2005). In Lee et al, study, insulin resistance has been recognized as the fundamental underlying metabolic defect in the pathogenesis of metabolic syndrome, a clustering of cardiovascular risk factors such as diabetes, hypertension, dyslipidemia, and obesity. Recent studies established that mitochondrial dysfunction is involved in insulin resistance in general and fetal origin of this state in particular. Because genes are the fundamental molecular basis of inheritance-and thus the cornerstones of evolution-a model explaining insulin resistance is based at the gene level at best. Since a certain mtDNA polymorphism, 1! 6189T>C, is associated with insulin resistance, mtDNA has to be a basic component of the gene-based model. It was developed a mitochondria-based model that explains insulin resistance in terms of quantitative and qualitative change of the mitochondrion and its DNA. This model can accommodate several important hypotheses, such as thrifty genotype hypothesis, thrifty phenotype hypothesis, fetal insulin hypothesis, contribution of metabolic imprinting by epigenetic changes, and many other features associated with insulin resistance (Lee et al., 2005). This suggests a connection among age-related refusing in mitochondrial function and insulin resistance

(Petersen et al., 2003). In the study of Petersen et al, the aim was to investigate how insulin resistance arises, it was studied healthy, lean, elderly and young participants matched for lean body mass and fat mass. Elderly study participants were markedly insulin-resistant as compared with young controls, and this resistance w! as attributable to reduced insulin-stimulated muscle glucose metabolism. These changes were associated with increased fat accumulation in muscle and liver tissue assessed by 1H nuclear magnetic resonance (NMR) spectroscopy, and with a approximately 40% reduction in mitochondrial oxidative and phosphorylation activity, as assessed by in vivo 13C/31P NMR spectroscopy. These data support the hypothesis that an age-associated decline in mitochondrial function contributes to insulin resistance in the elderly (Petersen et al., 2003).

OXIDATIVE STRESS, ANTIOXIDANTS AND DIABETES

Antioxidants are substances that inhibit the destructive effects of oxidation. Some of the general antioxidants that are known are glutathione reductase, glutathione peroxidase, glutathione, vitamins A, C, E, catalase, and enzyme superoxide dismutase. Treatment plans that are also associated with antioxidants are: physical exercise, insulin therapy, and antioxidant therapy.

Glutathione

Glutathione works as a direct free radical searcher, as a cosubstrate for

glutathione peroxidase, and as a cofactor for many enzymes, and develops the combination in endo- and xenobiotic reactions. There is contradiction in some statistics of increased glutathione dilution in diabetic rat kidney (Mekinova et al., 1995) and lens (Borenshtein et al., 2001). In Mekinova et al, research it was studied the effect of supplementation with vitamins C, E and beta-carotene on antioxidative status in kidneys of male Wistar rats with diabetes induced by intravenous application of streptozotocin. The animals received subtherapeutic doses of Insulin Interdep. A considerable decrease of malondialdehyde, reduced and oxidized glutathione and reduction of the activities of Se-glutathione peroxidase and glutathione S-transferase were observed in kidneys of diabetic rats treated with these vitamins. On the contrary, the activity of CuZn-superoxide dismutase and the ! level of vitamin C increased significantly. No changes were observed for vitamin E, beta-carotene and catalase. Supplementation with vitamins C, E and beta-carotene resulted in an improvement of antioxidative status of kidneys of rats with streptozotocin-induced diabetes (Mekinova et al., 1995). In the study of Borenshtein et al, diabetes commonly leads to long-term complications such as cataract. This study investigated the effects of alpha-lipoic acid (LPA) and its gamma-linolenic acid (GLA) conjugate on cataract development in diabetic sand rats. Two separate experiments were conducted. In Experiment 1, sand rats were fed a "high-energy" diet (70% starch), an acute model of Type 2 diabetes, and injected with LPA. In Experiment 2, the animals received a "medium-energy" diet (59% starch), a chronic diabetic model, and were intubated with LPA or its GLA conjugate. Throughout the experiments, blood glucose levels and cataract development were measured. At the termination of ! the experiments, lens aldose reductase (AR) activity and lenticular reduced glutathione (GSH) levels were

analyzed. LPA injection significantly inhibited cataract development and reduced blood glucose levels in rats fed the "high-energy" diet. Lens AR activity tended to be lower, while lenticular GSH levels increased. In sand rats fed a "medium-energy" diet (59% starch), LPA intubation had no effect on blood glucose levels and cataract development but GSH levels were increased. In contrast, sand rats intubated with GLA conjugate showed the highest blood glucose levels and accelerated cataract development. The conjugate treatment also decreased lenticular GSH content. The hypoglycemic effects of LPA are beneficial in the prevention of acute symptoms of Type 2 diabetes. It remains to be shown that the antioxidant activity of LPA is responsible for prevention or inhibition of cataract progression in sand rat (Borenshtein et al., 2001).

Glutathione reductase and peroxidase are two enzymes that originate in the cytoplasm, nucleus and mitochondria. The activity of glutathione reductase renews cellular glutathione is lowered in retina (Obrosova et al., 2000) and plasma, and higher in the heart (Rauscher et al., 2001). In the study of Obrosova et al, the study was designed to (1) evaluate retinal lipid peroxidation in early diabetes by the method specific for free malondialdehyde and 4-hydroxyalkenals, (2) identify impaired antioxidative defense mechanisms and (3) assess if enhanced retinal oxidative stress in diabetes is prevented by the potent antioxidant, DL-alpha-lipoic acid. The groups included control and streptozotocin-diabetic rats treated with or without DL-alpha-lipoic acid (100 mg kg(-1) day(-1), i.p., for 6 weeks). All parameters were measured in individual retina. 4-Hydroxyalkenal concentrations were increased in diabetic rats (2.63+/-0.60 vs. 1.44+/-0.30 nmol/mg soluble p! rotein in controls, $P<0.01$), and this increase was prevented by DL-alpha-lipoic acid (1.20+/-0.88, $P<0.01$ vs. untreated diabetic group).

Malondialdehyde, reduced glutathione (GSH) and oxidized glutathione (GSSG) concentrations were similar among the groups. Superoxide dismutase, glutathione peroxidase (GSHPx), glutathione reductase (GSSGRed) and glutathione transferase (GSHTrans) activities were decreased in diabetic rats vs. controls. Quinone reductase was upregulated in diabetic rats, whereas catalase and cytoplasmic NADH oxidase activities were unchanged. DL-alpha-Lipoic acid prevented changes in superoxide dismutase and quinone reductase activities induced by diabetes without affecting the enzymes of glutathione metabolism. In conclusion, accumulation of 4-hydroxyalkenals is an early marker of oxidative stress in the diabetic retina. Increased lipid peroxidation occurs in the absence of GSH depletion, and is prevented by DL-alpha-lipoic acid (Obrosova et al., 2000). In ! the study of Rauscher et al, Coenzyme Q10 is an endogenous lipid soluble antioxidant. Because oxidant stress may exacerbate some complications of diabetes mellitus, this study investigated the effects of subacute treatment with exogenous coenzyme Q10 (10 mg/kg/day, i.p. for 14 days) on tissue antioxidant defenses in 30-day streptozotocin-induced diabetic Sprague-Dawley rats. Liver, kidney, brain, and heart were assayed for degree of lipid peroxidation, reduced and oxidized glutathione contents, and activities of catalase, superoxide dismutase, glutathione peroxidase, and glutathione reductase. All tissues from diabetic animals exhibited increased oxidative stress and disturbances in antioxidant defense when compared with normal controls. Treatment with the lipophilic compound coenzyme Q10 reversed diabetic effects on hepatic glutathione peroxidase activity, on renal superoxide dismutase activity, on cardiac lipid peroxidation, and on oxidized glutathione concentration in brain. However, treatment with coenzyme Q10 also exacerbated the increase! in cardiac catalase activity, which was already

elevated by diabetes, further decreased hepatic glutathione reductase activity, augmented the increase in hepatic lipid peroxidation, and further increased glutathione peroxidase activity in the heart and brain of diabetic animals. Subacute dosing with coenzyme Q10 ameliorated some of the diabetes-induced changes in oxidative stress. However, exacerbation of several diabetes-related effects was also observed (Rauscher et al., 2001). Glutathione peroxidase metabolizes hydrogen peroxide to water with lowered glutathione as a hydrogen donor (Santini et al., 1997). In Santini et al, study, oxidative stress is postulated to be increased in patients with IDDM. Accumulating evidence suggests that oxidative cell injury caused by free radicals contributes to the development of IDDM complications. On the other side, a decreased efficiency of antioxidant defenses (both enzymatic and nonenzymatic) seems to correlate with the severity of ! pathological tissue changes in IDDM. Thus, it was determined plasma antioxidant defenses, measuring the total radical-trapping antioxidant capacity (TRAP) and the two markers of oxidative stress, lipid hydroperoxides (ROOHs) and conjugated dienes, in 72 patients with well-controlled IDDM and without evident complications, compared with 45 nondiabetic subjects. Compared with control subjects, IDDM patients showed significantly reduced plasma TRAP (669 +/- 131 vs. 955 +/- 104 micromole/l, $P < 0.001$) and significantly increased levels of ROOHs (7.13 +/- 2.11 vs. 2.10 +/- 0.71 micromole/l, $P < 0.001$) and conjugated dienes (0.0368 +/- 0.0027 vs. 0.0328 +/- 0.0023 arbitrary units [AU], $P < 0.01$), especially in the trans-trans conformation (0.0340 +/- 0.0028 vs. 0.0259 +/- 0.0022 AU, $P < 0.001$), with a concurrent reduction of conjugated dienes in the cis-trans conformation (0.0028 +/- 0.0011 vs. 0.0069 +/- 0.0012 AU, $P < 0.001$). The oxidative parameters studied did not appear to be correlated with metabolic control (HbA1c levels)

and lipid profile (c! holesterol or triglyceride levels). The reduced TRAP and the increased ROOH and conjugated diene plasma levels, together with the decreased ratio of cis-trans/trans-trans conjugated dienes, which reflects an altered redox status of plasma, indicate that in IDDM patients, oxidative stress is enhanced and antioxidant defenses are defective, regardless of diabetes duration, metabolic control, or presence of complications (Santini et al., 1997).

Vitamins

Administration of the antioxidants, for example the vitamin C and free amino acids, gets a better reaction to insulin and can supply extra benefits to the proposed reduction of oxidative stress in tissues {Paolisso, 1994 #29; Natarajan Sulochana, 2002 #31; }. In Paolisso et al, study, in aged healthy (n = 10) and non-insulin-dependent (type II) diabetic (n = 10) subjects matched for age [67.3 +/- 0.5 vs. 68.0 +/- 0.4 yr, P = not significant (NS)], body mass index (25.7 +/- 0.7 vs. 26.0 +/- 0.2 kg/m2, P = NS), gender ratio [6 males (M)/4 females (F) vs. 5 M/5 F], and mean arterial blood pressure (104 +/- 6 vs. 105 +/- 9 mmHg, P = NS), it was determined the changes in insulin secretion and action after vitamin C infusion and the relative increase in plasma vitamin C levels. At the highest vitamin C infusion rate (0.9 mmol/min) the increase in plasma vitamin C levels did not affect B cell response to glucose, but it improved Conard's K values and wh! ole body glucose disposal in healthy subjects and in diabetic patients. In both groups of subject vitamin C-mediated increase in insulin action was mainly due to an improvement in nonoxidative glucose metabolism. After fasting, plasma vitamin C levels

correlated with basal whole body glucose disposal ($r = -0.44$, $P < 0.05$; $n = 20$). After vitamin C infusion, percent change in plasma vitamin C level correlated with the percent decline in membrane microviscosity ($r = 0.53$, $P < 0.01$; $n = 20$) and increase in whole body glucose disposal ($r = 0.63$, $P < 0.003$; $n = 20$). In conclusion, plasma vitamin C levels seem to play a role in the modulation of insulin action in aged healthy and diabetic subjects (Paolisso et al., 1994). In the study of Natarajan Sulochana et al, oral amino acid intake reduces plasma glucose in Streptozotocin-induced diabetic rats. This study examined the effect of oral amino acid supplementation in patients with type 2 diabetes mellitus (DM). A double blind pilo! t clinical trial was conducted for a period of 2 months on 77 subjects with type 2 DM. Subjects of both sexes, ages 30-60, were included in the trial. All were receiving oral antidiabetic tablets. They were divided into groups on the basis of oral supplementation: (A) lysine, (B) essential amino acids, (C) amino acids and vitamins (fat and water-soluble), and (D) calcium phosphate (control). The subjects were periodically examined for fasting and post-prandial plasma glucose, fasting and post-prandial immunoreactive insulin, plasma amino acids, glycosylated hemoglobin (HbA1c), proteins and albumin in serum, urea and creatinine in plasma and sugar, and proteins and ketones in urine. The results revealed a significant decrease in PP plasma glucose ($P<0.05$) in group B when compared to groups C and D after 45 days. Plasma Arginine was increased in group C from 3.84 to 9.24 mg/dl. There were no statistically significant changes seen in other parameters between groups and visits. Oral supplementation with amino acids for patients with type 2 DM appe! ars to decrease PP plasma glucose without any change in plasma insulin levels, perhaps due to improved insulin sensitivity. However, the long-term effects of amino acids need further study

(Natarajan Sulochana et al., 2002). Vitamin C is a significant antioxidant for the defense of plasma lipids and will necessitate supplementation in circumstances of frequent provident situations, for instance hyperglycemia.

The supplementation of vitamin C unaccompanied demonstrates incomplete therapeutic advantages in type 1 diabetes (Je et al., 2001) and is more commonly used with vitamin E. In the study of Je et al, it was measured the plasma glucose and the glycosylated hemoglobin at the time of sacrifice in streptozotocin-induced diabetic mellitus (DM) rats. In diabetic rats, plasma glucose and glycosylated hemoglobin was increased as compared with normal rats, and vitamin E inhibited the increase of glycosylated hemoglobin level but vitamin C had no effect. The peroxidized proteins and lipids from the diabetic organs such as liver or kidney were measured to assess the oxidative damage. The 2,4-dinitrophenyl-hydrazine (DNPH) incorporation method was used to measure the peroxidized protein. In diabetic rats, DNPH incorporation was increased as compared with normal rats and vitamin E also inhibited the increase of DNPH incorporation but vitamin C had no effect. It s! uggests that the protein oxidation occurred on the liver in diabetic rats and the oxidative stress is general in the diabetic condition. It was measured the systolic arterial pressure and mean arterial pressure in normal rats, nephrectomy (NEPH)-rats, diabetic rats (DM), and NEPH-diabetic rats (NEPH-DM). Blood pressure was significantly increased in DM and NEPH-DM as compared with normal rats. In conclusion, plasma glucose, glycosylated hemoglobin, and the oxidation of proteins or lipid were increased in diabetic rats. Vitamin E decreased the plasma glucose, glycosylated hemoglobin and the oxidation of proteins and lipid, but vitamin C had no effects (Je et al., 2001). Nutritional vitamin E supplementation helps

fatty acids metabolism and lowers the lipid peroxidation in tissues of rats with diabetes (Celik et al., 2002) and increase the blood flow and nerve morphometric parameters in the heart (Rosen et al., 1995). In the study of Celik et al, the aim was to determine whet! her vitamin E supplementation in streptozotocin-induced diabetic rats treated with insulin could affect the levels of fatty acid composition and malondialdehyde (MDA) of brain, liver and muscle tissues. Thirty Wistar albino rats were used during the experiments. They were randomly divided into three groups, each consisting of six individuals. The first group was diabetic, the second was control, and the third was diabetic but fed vitamin E. The level of stearic acid in brain tissues decreased ($p<0.05$) in the second and the third groups as compared to the first group. The percentage of arachidonic and polyunsaturated fatty acids slightly decreased ($p<0.05$) in the diabetic group in comparison to the second and third groups. The proportion of docosahexaenoic acid significantly increased ($p<0.01$) in the second and third groups in contrast to the first group. The level of docosatrienoic was slightly higher ($p<0.05$) in the third group than in other groups. In the liver tissues, the proportion of stearic, oleic and total monounsaturated fatty acids w! as slightly higher ($p<0.05$) in the first group than in the other groups. The level of arachidonic, docosahexaenoic, and unsaturated and total polyunsaturated fatty acid slightly increased ($p<0.05$) in the second and third groups as compared to the first group. The level of myristic and stearic acids in muscle tissue slightly increased ($p<0.05$) in the first group as compared to the second and third groups. The proportion of arachidonic, docosahexenoic and unsaturated fatty acids slightly increased ($p<0.05$) in the second and third groups relative to the first group. The amount of MDA was slightly higher in the diabetic group than in the other groups in all tissues.

The results indicate that vitamin E supplementation, in experimental diabetes could play a role in controlling the oxidative status and altered fatty acid metabolism in tissues, thereby maintaining favorable fatty acid distribution in the tissues affected by diabetic complications (Celik et al., 2002). In the study ! of Rosen et al, increased oxidative stress has been suggested to contribute to disturbances in the regulation of coronary flow and the increased cardiac risk in diabetes mellitus. Using the isolated perfused heart of streptozotocin-diabetic rats our study shows that basal and maximal coronary flow (tested by infusion of sodium nitroprusside) are not altered in diabetes, but that 5-hydroxytryptamine (5-HT) stimulated endothelium-dependent increase in coronary flow becomes progressively impaired. This defect of the endothelium-dependent vasodilatation was prevented by perfusion of the hearts with superoxide dismutase and pretreatment of the diabetic rats with tocopherol-acetate. Morphological studies also revealed that pretreatment with tocopherol-acetate was cardioprotective, and largely prevented severe alterations of myocardial structure typically observed after a diabetes duration of 3 months; deterioration and fragmentation of myofilament bundles were seen less, and the numbers of areas of focal necrosis and of contraction bands were clearl! y reduced. In contrast to untreated diabetic hearts the autonomic nerve fibers detected by catecholamine fluorescence were running in parallel in hearts of tocopherol-treated diabetic rats, and the amount of catecholamines was not different from that of healthy control rats. Trichrome staining and immunohistochemical staining of collagen I and III showed a dramatic increase in number and the size of deposits of collagen fibers at precapillary locations in the diabetic hearts, which were significantly reduced by anti-oxidative treatment. These findings demonstrate that oxidative stress may not only play a

major role in the impairment of endothelium-dependent regulation of coronary flow, but also in the development of perivascular fibrosis and severe changes of the autonomic nerves and contractile system in myocardium (Rosen et al., 1995).

Oral vitamin C and vitamin E has the ability to lower the oxidative stress in the eye (Peponis et al., 2002) and the vascular endothelial function gets better in type 1 but not type 2 (Beckman et al., 2003). In the study of Peponis et al, to investigate the effect of vitamin C and E supplementation in the levels of nitrite, nitric oxide (NO) related metabolite, and ocular surface parameters in diabetic patients. 50 patients with non-insulin dependent diabetes mellitus were given vitamin C (1000 mg/day) and vitamin E (400 IU/day) supplementation for 10 days. Nitrite levels in tears were measured by photometric determination before and after vitamin supplementation. Tear function parameters (Schirmer test I, BUT, ocular ferning test) and brush cytology analysis of the conjunctival epithelium were also evaluated. Nitrite levels were found to be significantly reduced ($p<0.05$) after 10 days of vitamin C and E supplementation. Improved values for Schirmer test, BUT test, and o! cular ferning test were also found. Goblet cell density and grading of squamous metaplasia showed a significant improvement. Oxidative stress and free radical production are elevated in diabetes mellitus. Antioxidants, such as vitamin C and vitamin E, probably have an important role in reducing the oxidative damage produced by nitric oxide and other free radicals and improving the ocular surface milieu (Peponis et al., 2002). In the study of Beckman et al, oxidative stress decreases the bioavailability of endothelium-derived nitric oxide in diabetic patients. It was investigated whether impaired endothelium-dependent vasodilatation (EDV) in diabetes can be improved by long-term administration of oral antioxidants. Forty-nine diabetic

subjects [26 Type 1 (T1) and 23 Type 2 (T2)] and 45 matched healthy control subjects were randomized to receive oral vitamin C (1,000 mg) and vitamin E (800 IU) daily or matching placebo for 6 mo. Vascular ultrasonography was used to determine ! brachial artery EDV and endothelium-independent vasodilatation (EIV). EDV was decreased in both T1 (4.9 +/- 0.9%, P = 0.015) and T2 (4.1 +/- 1.0%, P < 0.01) subjects compared with control subjects (7.7 +/- 0.7%). EIV was decreased in T2 (15.0 +/- 1.2%, P < 0.01) but not T1 subjects (18.5 +/- 2.3%, P = 0.3) compared with controls (21.8 +/- 1.8%). Administration of antioxidant vitamins increased EDV in T1 (by 3.4 +/- 1.4%, P = 0.023) but not T2 subjects (by 0.5. +/- 0.4%, P = 0.3). Antioxidant therapy had no significant affect on EIV. Oral antioxidant therapy improves EDV in T1 but not T2 diabetes. These results are consistent with the lack of clinical benefit in studies that have included primarily T2 diabetic patients (Beckman et al., 2003). Analysis of various clinical tests examining vitamin E therapy had shown that large amounts of vitamin E (more than 400 IU per day) does damage and it can actually cause death (Miller et al., 2005). In Miller et al, study experimental models and observational studies suggest that vitamin E supplementation ! may prevent cardiovascular disease and cancer. However, several trials of high-dosage vitamin E supplementation showed non-statistically significant increases in total mortality. The purpose of this study was to perform a meta-analysis of the dose-response relationship between vitamin E supplementation and total mortality by using data from randomized, controlled trials. There were 135,967 participants in 19 clinical trials. Of these trials, 9 tested vitamin E alone and 10 tested vitamin E combined with other vitamins or minerals. The dosages of vitamin E ranged from 16.5 to 2000 IU/d (median, 400 IU/d). The information was obtained from Pub Med from 1966 through

August 2004, complemented by a search of the Cochrane Clinical Trials Database and review of citations of published reviews and meta-analyses. No language restrictions were applied. The synthesis data was: 9 of 11 trials testing high-dosage vitamin E (> or =400 IU/d) shown increased risk (risk difference > 0) for a! ll-cause mortality in comparisons of vitamin E versus control. The pooled all-cause mortality risk difference in high-dosage vitamin E trials was 39 per 10,000 persons (95% CI, 3 to 74 per 10,000 persons; P = 0.035). For low-dosage vitamin E trials, the risk difference was -16 per 10,000 persons (CI, -41 to 10 per 10,000 persons; P > 0.2). A dose-response analysis showed a statistically significant relationship between vitamin E dosage and all-cause mortality, with increased risk of dosages greater than 150 IU/d. High-dosage (> or =400 IU/d) trials were often small and were performed in patients with chronic diseases. The generalizability of the findings to healthy adults is uncertain. Precise estimation of the threshold at which risk increases is difficult. High-dosage (> or =400 IU/d) vitamin E supplements may increase all-cause mortality and should be avoided (Miller et al., 2005). Some facts show that both vitamin C and vitamin E have no effect in reducing biomarkers of oxidative stress. Willems et al. (Willems et al., 1998) found that the! principles of antioxidant stage, vitamin A or vitamin E that were not at a low level in well controlled in fragile type 1 diabetic patients and there were not found dissimilarities distinguished between patients categorized by sub-clinical complications. It was found in a pediatric study that antioxidant activity was reduced when compared to the inadequate glycemic control (Asayama et al., 1993). In the study of Asayama et al, the intention was to determine whether alteration in serum antioxidant status is related to the increased oxidative stress as a cause of diabetic angiopathy. It was measured both the antioxidant

activity and total peroxyl radical-trapping antioxidant parameter, and their component individual antioxidants in serum of children with insulin-dependent diabetes mellitus. Antioxidants measured were ceruloplasmin, transferrin, and albumin as components of antioxidant activity; and ascorbic acid, uric acid, protein sulfhydryl, and alpha-tocopherol as compone! nts of total peroxyl radical-trapping antioxidant parameter. Serum antioxidant activity appeared to be decreased in the diabetics in relation to poor glycemic control, corresponding to the decrease in transferrin and albumin. Serum haptoglobin level was also decreased in the diabetics. Similarly, the directly measured TRAP value was decreased in the diabetic serum mainly due to the decreased contribution of unidentified chain-breaking antioxidants, despite the increase in ascorbic acid and alpha-tocopherol. The decrease in both types of antioxidant activity in the diabetic serum, as new findings, suggests that a defective serum antioxidant status contributes to the increased oxidative stress in insulin-dependent diabetes mellitus (Asayama et al., 1993).

Catalase

Catalase is found in peroxisomes, and breaks down hydrogen peroxide to oxygen and water. Modifications of catalase actions because of diabetes are regulated by treatment with captopril (Kedziora-Kornatowska et al., 2000), aminoguanidine (Kedziora-Kornatowska et al., 1998), melatonin, acetylsalicylic acid (Caballero et al., 2000), DHEA (dehydroepiandrosterone) (Aragno et al., 1999), probucol {Kaul, 1995 #80; Kaul, 1996 #81}, and stobadine (Stefek et al., 2000), all of them were administrated ahead of or in the same time while the diabetogen. In the study of Kedziora-Kornatowska

et al, effects of the angiotensin convertase inhibitors captopril (CAP) and enalapril (ENA) on the malondialdehyde (MDA) content and the activities of superoxide dismutase (SOD) and catalase in the kidneys of rats with streptozotocin-induced diabetes were studied. Induction of diabetes resulted in an increase of MDA concentration and progressive decreases of SOD and catalase activities after ! 6 and 12 weeks. CAP and ENA administration did not affect body weight changes or blood glucose and HbA1c contents in diabetic rats but decreased albuminuria and kidney weight increase, attenuated lipid peroxidation, and prevented the decreases in SOD and catalase activities. These results confirm the oxidative stress in streptozotocin-induced experimental diabetes and point to the beneficial antioxidant effects of angiotensin convertase inhibitors (Kedziora-Kornatowska et al., 2000). In the study of Kedziora-Kornatowska et al, the effect of aminoguanidine (AG) on the malondialdehyde (MDA) concentration and activities of superoxide dismutase (SOD), catalase and glutathione peroxidase (GSH-Px) in erythrocytes of rats with streptozotocin-induced diabetes was studied. Induction of diabetes resulted in an increase of MDA concentration and decreases of SOD and catalase activities after 6 and 12 weeks. GSH-Px activity increased after 6 weeks and returned to control values after 12! weeks. AG administration did not affect body weight, blood glucose level and HbA1c content in diabetic rats but led to a decrease of MDA concentration and SOD and catalase activities after 12 weeks of treatment, with no significant effect after 6 weeks. AG attenuated the GSH-Px increase after 6 weeks but augmented the activity of this enzyme after 12 weeks. These results confirm the presence of oxidative stress in streptozotocin-induced experimental diabetes and point to the beneficial antioxidant effect of AG (Kedziora-Kornatowska et al., 1998). In Caballero et

al, study, aspirin inhibits protein glycation by acetylation of free amino groups. In the diabetic status, it was demonstrated that several enzymes of heme pathway were diminished. The aim of this work has been to investigate the in vivo effect of short and long term treatment with acetylsalicylic acid in streptozotocin induced diabetic mice. In both treatments, the acetylsalicylic acid prevented delta-aminolevulinic dehydratase and porphobilinogen deaminase inactivation in diabetic m! ice and blocked the accumulation of lipoperoxidative aldehydes. Catalase activity was significantly augmented in diabetic mice and the long term treatment with aspirin partially reverted it. It was proposed that oxidative stress might play an important role in streptozotocin induced diabetes. The results suggest that aspirin can prevent some of the late complications of diabetes, lowering glucose concentration and probably inhibiting glycation by acetylation of protein amino groups (Caballero et al., 2000). In the study of Aragno et al, chronic hyperglycemia in diabetes determines the overproduction of free radicals, and evidence is increasing that these contribute to the development of diabetic complications. It has recently been reported that dehydroepiandrosterone possesses antioxidant properties; this study evaluates whether, administered daily for three weeks per os, it may provide antioxidant protection in tissues of rats with streptozotocin-induced diabetes. Lipid pe! roxidation was evaluated on liver, brain and kidney homogenates from diabetic animals, measuring both steady-state concentrations of thiobarbituric acid reactive substances and fluorescent chromolipids. Hyperglycemic rats had higher thiobarbituric acid reactive substances formation and fluorescent chromolipids levels than controls. Dehydroepiandrosterone-treatment (4 mg/day for 3 weeks) protected tissues against lipid peroxidation: liver, kidney and brain homogenates from dehydroepiandrosterone-treated

animals showed a significant decrease of both thiobarbituric acid reactive substances and fluorescent chromolipids formation. The effect of dehydroepiandrosterone on the cellular antioxidant defenses was also investigated, as impaired antioxidant enzyme activities were considered proof of oxygen-dependent toxicity. In kidney and liver homogenates, dehydroepiandrosterone treatment restored to near-control values the cytosolic level of reduced glutathione, as well as the enzymatic activities of superoxide-dismutase, glutathione-peroxidase, and cat! alase. In the brain, only an increase of catalase activity was evident ($p < .05$), which reverted with dehydroepiandrosterone treatment. The results demonstrate that DHEA treatment clearly reduces oxidative stress products in the tissues of streptozotocin-treated rats (Aragno et al., 1999). In the study of Kaul et al, earlier it was reported that probucol treatment subsequent to the induction of diabetes could prevent diabetes-associated changes in myocardial antioxidants as well as function at 8 weeks. In this study, it was examined the efficacy of probucol in the reversal of diabetes induced myocardial changes. Rats were made diabetic with a single injection of streptozotocin (65 mg/kg, i.v.). After 4 weeks of induction of diabetes, a group of animals was treated on alternate days with probucol (10 mg/kg i.p.), a known lipid-lowering agent with antioxidant properties. At 8 weeks, there was a significant drop in the left ventricle (LVSP) and aortic systolic pressures (ASP) ! in the diabetic group. Hearts from these animals showed an increase in the thiobarbituric acid reacting substances (TBARS), indicating increased lipid peroxidation. This was accompanied by a decrease in the myocardial antioxidant enzymes activities, superoxide dismutase (SOD) and glutathione peroxidase (GSHPx). Myocardial catalase activity in the diabetic group was higher. In the diabetic + probucol group both LVSP and ASP showed significant recovery. This was

also accompanied by an improvement in SOD and GSHPx activities and there was further increase in the catalase activity. Levels of the TBARS were decreased in this group. These data provide evidence that diabetic cardiomyopathy is associated with an antioxidant deficit which can be reversed with probucol treatment. Improved cardiac function with probucol may be due to the recovery of antioxidants in the heart (Kaul et al., 1996). In Stefek et al, study, the purpose of the present study was to investigate the effect of dietary supplementation with the pyridoindole antioxidant stobadine on ! the myocardial antioxidant status and ultrastructure of streptozotocin-diabetic rats. Diabetic male Wistar rats were fed for 32 weeks a standard diet or a diet supplemented with stobadine (0.05% w/w). Control rats received a standard diet or stobadine-supplemented diet (0.16% w/w). Plasma levels of glucose, cholesterol and triglycerides were increased significantly by diabetes. Activities of superoxide dismutase and catalase were markedly elevated in the diabetic myocardium. Myocardial levels of conjugated dienes increased after eight months of diabetes, in spite of significantly increased myocardial alpha-tocopherol and coenzyme Q9 content. The long-term treatment of diabetic animals with stobadine (i) reduced plasma cholesterol and triglyceride levels yet left the severe hyperglycemia unaffected, (ii) reduced oxidative damage of myocardial tissue as measured by conjugated dienes, (iii) reversed myocardial levels of alpha-tocopherol and coenzyme Q9 to near control values, ! (iv) reduced elevated activity of superoxide dismutase in the diabetic myocardium, and (v) attenuated angiopathic and atherogenic processes in the myocardium as assessed by electron microscopy examination. These results are in accordance with the postulated prooxidant role of chronic hyperglycemia and provide further evidence that development of pathological changes in diabetic

myocardium is amenable to pharmacological intervention by biological antioxidants (Stefek et al., 2000).

The effects of diabetes on cardiac catalase activity are aggravated by the treatment with quercetin (Sanders et al., 2001). In the study of Sanders et al, in the light of evidence that some complications of diabetes mellitus may be caused or exacerbated by oxidative damage, it was investigated the effects of subacute treatment with the antioxidant quercetin on tissue antioxidant defense systems in streptozotocin-induced diabetic Sprague-Dawley rats (30 days after streptozotocin induction). Quercetin, 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-1-benzopyran-4-one, was administered at a dose of 10mg/kg/day, ip for 14 days, after which liver, kidney, brain, and heart were assayed for degree of lipid peroxidation, reduced and oxidized glutathione content, and activities of the free-radical detoxifying enzymes catalase, superoxide dismutase, glutathione peroxidase, and glutathione reductase. Treatment of normal rats with quercetin increased serum AST an! d increased hepatic concentration of oxidized glutathione. All tissues from diabetic animals exhibited disturbances in antioxidant defense when compared with normal controls. Quercetin treatment of diabetic rats reversed only the diabetic effects on brain oxidized glutathione concentration and on hepatic glutathione peroxidase activity. By contrast, a 20% increase in hepatic lipid peroxidation, a 40% decline in hepatic glutathione concentration, an increase in renal (23%) and cardiac (40%) glutathione peroxidase activities, and a 65% increase in cardiac catalase activity reflect intensified diabetic effects after treatment with quercetin. These results call into question the ability of therapy with the antioxidant quercetin to reverse diabetic oxidative stress in an overall sense (Sanders et al., 2001).

Superoxide dismutase

Superoxide dismutase is restrained in the mitochondria, yet it can be released into extracellular space (Reiter et al., 2000). In the review written by Reiter et al, it is specified that melatonin was discovered to be a direct free radical scavenger less than 10 years ago. Besides its ability to directly neutralize a number of free radicals and reactive oxygen and nitrogen species, it stimulates several antioxidative enzymes that increase its efficiency as an antioxidant. In terms of direct free radical scavenging, melatonin interacts with the highly toxic hydroxyl radical with a rate constant equivalent to that of other highly efficient hydroxyl radical scavengers. Additionally, melatonin reportedly neutralizes hydrogen peroxide, singlet oxygen, peroxynitrite anion, nitric oxide and hypochlorous acid. The following antioxidative enzymes are also stimulated by melatonin: superoxide dismutase, glutathione peroxidase and glutathione reductase. Melaton! in has been widely used as a protective agent against a wide variety of processes and agents that damage tissues via free radical mechanisms (Reiter et al., 2000). The activity in the aorta that did not affect diabetes may be high (El-Khatib et al., 2001) or may be low in red blood cells, low in retina (Obrosova et al., 2000) and plasma, and high in pancreas (Jang et al., 2000). In the study of El-Khatib et al, the effects of aminoguanidine (AG; 100 mg x kg(-1)) and desferrioxamine (DFO; 50 mg x kg(-1)) on some vascular and biochemical changes associated with streptozotocin (STZ; 65 mg x kg(-1); i.p.)-induced hyperglycemia was investigated in rats. Both AG and DFO were administered i.p., once daily, for 14 consecutive days to normal and hyperglycemic

animals. The responsiveness of the isolated aortic rings to phenylephrine (PE) was tested. In addition, biochemical markers for oxidative stress such as plasma levels of lipid peroxides and total thiols, as well as the activiti! es of erythrocytic superoxide dismutase (SOD) and whole blood glutathione peroxidase (GSH-Px) were assessed. Results of the present study indicated that induction of hyperglycemia was associated with increased aortic ring responsiveness to PE, loss in body weight, increase in urine volume, elevation of plasma total thiols and lipid peroxide levels and elevated SOD and GSH-Px enzymatic activities. Treatment of normal rats with AG reduced the response of their aorta to PE. Furthermore, a profound increase in body weight without any significant change in the measured biochemical parameters was observed. In hyperglycemic animals, AG tended to normalize the enhanced aortic response to PE and modulated STZ-induced biochemical changes without affecting the elevated plasma glucose level. Treatment of normal rats with DFO reduced the response of their aorta to PE and decreased their body weight without altering any of the chosen biochemical parameters. In hyperglycemic animals, DFO attenuated the responsiveness of their aorta to PE and at the same time! , did not affect the loss in body weight and the elevation of plasma glucose level observed in the hyperglycemic group. Additionally, DFO normalized the elevated plasma level of total thiols and exerted a modulatory influence on the enhanced activities of SOD and GSH-Px as well as on the increased levels of lipid peroxides. The data lend further credence for the contribution of oxidative stress in the vascular and biochemical changes associated with STZ-induced hyperglycemia. It is also apparent that advanced glycosylation end products and nitric oxide might be involved. Until clinical studies prove the efficacy and safety of these drugs, specific agents that could scavenge free

radicals and block protein glycosylation seem beneficial as a helpful adjunct to the therapy of diabetes (El-Khatib et al., 2001). In the study of Obrosova et al, the aim of this study was designed to (1) evaluate retinal lipid peroxidation in early diabetes by the method specific for free malondial! dehyde and 4-hydroxyalkenals, (2) identify impaired antioxidative defense mechanisms and (3) assess if enhanced retinal oxidative stress in diabetes is prevented by the potent antioxidant, DL-alpha-lipoic acid. The groups included control and streptozotocin-diabetic rats treated with or without DL-alpha-lipoic acid (100 mg kg(-1) day(-1), i.p., for 6 weeks). All parameters were measured in individual retina. 4-Hydroxyalkenal concentrations were increased in diabetic rats (2.63+/-0.60 vs. 1.44+/-0.30 nmol/mg soluble protein in controls, $P<0.01$), and this increase was prevented by DL-alpha-lipoic acid (1.20+/-0.88, $P<0.01$ vs. untreated diabetic group). Malondialdehyde, reduced glutathione (GSH) and oxidized glutathione (GSSG) concentrations were similar among the groups. Superoxide dismutase, glutathione peroxidase (GSHPx), glutathione reductase (GSSGRed) and glutathione transferase (GSHTrans) activities were decreased in diabetic rats vs. controls. Quinone reductase was upregulated in diabetic rats, whereas catalase and cytoplasmic NADH oxidase! activities were unchanged. DL-alpha-Lipoic acid prevented changes in superoxide dismutase and quinone reductase activities induced by diabetes without affecting the enzymes of glutathione metabolism. In conclusion, accumulation of 4-hydroxyalkenals is an early marker of oxidative stress in the diabetic retina. Increased lipid peroxidation occurs in the absence of GSH depletion, and is prevented by DL-alpha-lipoic acid (Obrosova et al., 2000). In the study of Jang et al, increased oxidative stress has been suggested to be involved in the pathogenesis and progression of diabetic tissue

damage. Several antioxidants have been described as beneficial for oxidative stress-associated diseases. Boldine ([s]-2,9-dihydroxy-1, 10-dimethoxyaporphine) is a major alkaloid found in the leaves and bark of boldo (Peumus boldus Molina), and has been shown to possess antioxidant activity and anti-inflammatory effects. From this point of view, the possible anti-diabetic effect of boldine and ! its mechanism were evaluated. The experiments were performed on male rats divided into four groups: control, boldine (100 mg kg(-1), daily in drinking water), diabetic [single dose of 80 mg kg(-1)of streptozotocin (STZ), i.p.] and diabetic simultaneously fed with boldine for 8 weeks. Diabetic status was evaluated periodically with changes of plasma glucose levels and body weight in rats. The effect of boldine on the STZ-induced diabetic rats was examined with the formation of malondialdehydes and carbonyls and the activities of endogenous antioxidant enzymes (superoxide dismutase and glutathione peroxidase) in mitochondria of the pancreas, kidney and liver. The scavenging action of boldine on oxygen free radicals and the effect on mitochondrial free radical production were also investigated. The treatment of boldine attenuated the development of hyperglycemia and weight loss induced by STZ injection in rats. The levels of malondialdehyde (MDA) and carbonyls in liver, kidney and pancreas mitochondria were significantly increased in STZ-treated ! rats and decreased after boldine administration. The activities of mitochondrial manganese superoxide dismutase (MnSOD) in the liver, pancreas and kidney were significantly elevated in STZ-treated rats. Boldine administration decreased STZ-induced elevation of MnSOD activity in kidney and pancreas mitochondria, but not in liver mitochondria. In the STZ-treated group, glutathione peroxidase activities decreased in liver mitochondria, and were elevated in pancreas and kidney mitochondria. The boldine treatment restored

the altered enzyme activities in the liver and pancreas, but not the kidney. Boldine attenuated STZ- and iron plus ascorbate-induced MDA and carbonyl formation and thiol oxidation in the pancreas homogenates. Boldine decomposed superoxide anions, hydrogen peroxides and hydroxyl radicals in a dose-dependent manner. The alkaloid significantly attenuated the production of superoxide anions, hydrogen peroxide and nitric oxide caused by liver mitochondria. The resul! ts indicate that boldine may exert an inhibitory effect on STZ-induced oxidative tissue damage and altered antioxidant enzyme activity by the decomposition of reactive oxygen species and inhibition of nitric oxide production and by the reduction of the peroxidation-induced product formation. Boldine may attenuate the development of STZ-induced diabetes in rats and interfere with the role of oxidative stress, one of the pathogeneses of diabetes (Jang et al., 2000). Modifications of superoxide dismutase activity in diabetic animals are regulated by probucol (Kaul et al., 1996), captopril (Kedziora-Kornatowska et al., 1998), DHEA (Aragno et al., 1999), melatonim, boldine (Jang et al., 2000), nitecapone (Lal et al., 2000), and stobadine (Stefek et al., 2000). In Lal et al, study, the development and progression of diabetic nephropathy is dependent on glucose homeostasis and many other contributing factors. In the present study, it was examined the effect of nitecapone, an inhibitor of the dopamine-metabolizing enzyme catechol-O-methyl transferase ! (COMT) and a potent antioxidant, on functional and cellular determinants of renal function in rats with streptozotocin-induced diabetes. Administration of nitecapone to diabetic rats normalized urinary sodium excretion in a manner consistent with the dopamine-dependent inhibition of proximal tubule Na,K-ATPase activity. Hyperfiltration, focal glomerulosclerosis, and albuminuria were also reversed by nitecapone, but in a manner that is more readily

attributed to the antioxidant potential of the agent. A pattern of elevated oxidative stress, measured as CuZn superoxide dismutase gene expression and thiobarbituric acid-reactive substance content, was noted in diabetic rats, and both parameters were normalized by nitecapone treatment. In diabetic rats, activation of glomerular protein kinase C (PKC) was confirmed by isoform-specific translocation and Ser23 phosphorylation of the PKC substrate Na,K-ATPase. PKC-dependent changes in Na,K-ATPase phosphorylation was associated with ! decreased glomerular Na,K-ATPase activity. Nitecapone-treated diabetic rats were protected from these intracellular modifications. The combined results suggest that the COMT-inhibitory and antioxidant properties of nitecapone provide a protective therapy against the development of diabetic nephropathy (Lal et al., 2000). All of them were taken prior to or associated with the diabetogen.

Antioxidants reserves

Several examinations are accessible for the measurement of the entire antioxidant potential in biological samples, as well as tissue and plasma (Benzie and Strain, 1996; Prior and Cao, 1999). In the review written by Prior et al, the use of peroxyl or hydroxyl radicals as pro-oxidants in the oxygen radical absorbance capacity assay makes it different and unique from the assays that involve oxidants that are not necessarily pro-oxidants (Prior and Cao, 1999). In the examination made by Benzie et al, a simple, automated test measuring the ferric reducing ability of plasma, the FRAP assay, is presented as a novel method for assessing "antioxidant power." Ferric to ferrous ion reduction at low pH causes a colored ferrous-tripyridyltriazine complex to form. FRAP

values are obtained by comparing the absorbance change at 593 nm in test reaction mixtures with those containing ferrous ions in known concentration. Absorbance changes are linear over a wide concentration range w! ith antioxidant mixtures, including plasma, and with solutions containing one antioxidant in purified form. There is no apparent interaction between antioxidants. Measured stoichiometric factors of Trolox, alpha-tocopherol, ascorbic acid, and uric acid are all 2.0; that of bilirubin is 4.0. Activity of albumin is very low. Within- and between-run CVs are <1.0 and <3.0%, respectively, at 100-1000 micromol/liter. FRAP values of fresh plasma of healthy Chinese adults: 612-1634 micromol/liter (mean, 1017; SD, 206; n = 141). The FRAP assay offers a putative index of antioxidant, or reducing, potential of biological fluids within the technological reach of every laboratory and researcher interested in oxidative stress and its effects (Benzie and Strain, 1996). The entire radical antioxidant assay shows that the individuals with diabetes have lesser antioxidants resistance and that entire antioxidant potential is an improved sign of antioxidant status than the assessment of indivi! dual antioxidants (Ceriello et al., 1997). In the study of Ceriello et al, the existence of an oxidative stress in diabetes is still debated. This is largely due to the lack of good tools to assay the level of oxidative stress. The use of total radical-trapping antioxidant parameter (TRAP) has recently been proposed to explore the antioxidant property of a plasma sample. TRAP may be either directly measured by a fluorescence-based method (TRAPm) or calculated (TRAPc) by a mathematical formula, taking into account the serum levels of four natural antioxidants: protein-bound SH (thiol) groups, uric acid, vitamin E, and vitamin C. The difference between TRAPm and TRAPc is due to antioxidants, which are still unidentified, and to the possible synergism among the antioxidants. In this study, it was

evaluated malondialdehyde (MDA), TRAPm, TRAPc, protein-bound SH groups, uric acid, vitamin E, and vitamin C in 40 NIDDM patients and 40 matched normal control subjects. TRAPm and TRAPc were significantly lower in diabetic patients. A good correlation bet! ween TRAPm and TRAPc was found in both NIDDM patients ($r = 0.68$, $P < 0.0001$) and control subjects ($r = 0.74$, $P < 0.0001$). Protein-bound SH groups and uric acid were significantly lower in diabetic subjects, while MDA and vitamin E level were significantly higher. After correction for serum triglycerides (MDA) and cholesterol (vitamin E), MDA lost significance, while vitamin E did not. Vitamin C was not different in the two groups. These data show decreased TRAP levels in NIDDM patients, suggesting the existence of lower antioxidant defenses in diabetes. The decrease appears to be due to various antioxidants, some of them not yet clearly defined. TRAP may represent a more reliable estimation of serum antioxidant capacity than the measurement of each known antioxidants. The correlation found between TRAPm and TRAPc values suggests that TRAPc, easier to measure than TRAPm, might be adequately reliable for routine assessment of oxidative stress in diabetic patients (Ceriello et! al., 1997).

Physical Exercise and Oxidative Stress in Diabetes

Exercising is a physical activity that includes any kind of physical movement. Physical exercise is good for diabetics because it reduces the oxidative injury. However, normal training appears to improve antioxidant resistance and it has the ability to reduce lipid peroxidation. Also, it reduces the sugar level, blood pressure and cholesterol, and increases the blood circulation and helps insulin work well in the body. In addition, it makes a person feel better. In the therapy of diabetes, exercise is most important.

Exercise helps in the control of diabetes and reduces the chances of dying from heart disease. Exercise in type 2 diabetes is good because of the following: the chances of heart disease are decreased, the avoidance of diabetes in those at a high risk, the muscles have grater sensitivity to insulin, the blood sugar control is improved, the blood cholesterol profile and the blood pressure control is better, the prospective of weight ! loss and the general sense of well being is improved. (The information from above was provided from "American Diabetes Association"). Clinical experimentation had proven that exercising or physical training affected insulin action in muscle and perhaps other tissues in a good, positive way (Borghouts and Keizer, 2000). In the review of Borghouts et al, physical activity has a beneficial effect on insulin sensitivity in normal as well as insulin resistant populations. A distinction should be made between the acute effects of exercise and genuine training effects. Up to two hours after exercise, glucose uptake is in part elevated due to insulin independent mechanisms, probably involving a contraction-induced increase in the amount of GLUT4 associated with the plasma membrane and T-tubules. However, a single bout of exercise can increase insulin sensitivity for at least 16 h post exercise in healthy as well as NIDDM subjects. Recent studies have accord! ingly shown that acute exercise also enhances insulin stimulated GLUT4 translocation. Increases in muscle GLUT4 protein content contribute to this effect, and in addition it has been hypothesized that the depletion of muscle glycogen stores with exercise plays a role herein. Physical training potentates the effect of exercise on insulin sensitivity through multiple adaptations found in glucose transport and metabolism. In addition, training may elicit favorable changes in lipid metabolism and can bring about improvements in the regulation of hepatic glucose output, which is especially relevant to NIDDM. It is

concluded that physical training can be considered to play an important, if not essential role in the treatment and prevention of insulin insensitivity (Borghouts and Keizer, 2000).

Exercise is the most influential agent that sensitizes tissues to insulin and through manipulating post-receptor insulin signaling and glucose transport (Wojtaszewski et al., 1999). In the study of Wojtaszewski et al, physical exercise promotes glucose uptake into skeletal muscle and makes the working muscles more sensitive to insulin. To understand the role of insulin receptor (IR) signaling in these responses, it was studied the effects of exercise and insulin on skeletal muscle glucose metabolism and insulin signaling in mice lacking insulin receptors specifically in muscle. Muscle-specific insulin receptor knockout (MIRKO) mice had normal resting 2-deoxy-glucose (2DG) uptake in soleus muscles but had no significant response to insulin. Despite this, MIRKO mice displayed normal exercise-stimulated 2DG uptake and a normal synergistic activation of muscle 2DG uptake with the combination of exercise plus insulin. Glycogen content and glycogen synthase activity in restin! g muscle were normal in MIRKO mice, and exercise, but not insulin, increased glycogen synthase activity. Insulin, exercise, and the combination of exercise plus insulin did not increase IR tyrosine phosphorylation or phosphatidylinositol 3-kinase activity in MIRKO muscle. In contrast, insulin alone produced a small activation of Akt and glycogen synthase kinase-3 in MIRKO mice, and prior exercise markedly enhanced this insulin effect. In conclusion, normal expression of muscle insulin receptors is not needed for the exercise-mediated increase in glucose uptake and glycogen synthase activity in vivo. The synergistic activation of glucose transport with exercise plus insulin is retained in MIRKO mice, suggesting a phenomenon mediated by nonmuscle cells or by downstream signaling events

(Wojtaszewski et al., 1999). Furthermore, it is being considered that physical activity plays a very important role in the management and the avoidance of insulin insensitivity. In a study! that has been made shows the contrary about physical exercise in which physical exercise is not that good for the body. Non-enzymatic glycation is concerned in the pathogenesis of different diseases such as Alzheimer's and diabetes mellitus during physiologic process of aging (Stoppa et al., 2006). In Stoppa et al, study, it was observed an increase in the production of reactive oxygen species could occur by non-enzymatic glycation and glucose autoxidation. Furthermore, the association of hyperglycemia with strenuous physical exercise may induce cellular damage by impairing the antioxidant defense system (Stoppa et al., 2006).

Insulin Therapy

Insulin therapy is very important for diabetics with diabetes type 2. In the past the only way a person could get insulin in their body was by syringe. Nowadays the insulin pump is available. Another way of getting insulin in the body is by inhalation, which is being researched now. The amount of insulin needed in the body consists of how much a person exercises and by how much a person sleeps. Good sugar control helps in preventing the diabetes complications. Insulin therapy is an essential requirement for people with diabetes type 1. Twenty seven percent of individuals with type 2 diabetes use insulin therapy and not even a half can achieve what is proposed A1C level of 7% (Koro et al., 2004). In the study of Koro et al, the purpose of the study was to describe the changes in demographics, antidiabetic treatment, and glycemic control among the

prevalent U.S. adult diagnosed type 2 diabetes population between the National Health and Nutrition Examination Survey (NHA! NES) III (1988-1994) and the initial release of NHANES 1999-2000. The study population was derived from NHANES III (n = 1,215) and NHANES 1999-2000 (n = 372) subjects who reported a diagnosis of type 2 diabetes with available data on diabetes medication and HbA(1c). Four therapeutic regimens were defined: diet only, insulin only, oral antidiabetic drugs (OADs) only, or OADs plus insulin. Multiple logistic regressions were used to examine changes in antidiabetic regimens and glycemic control rates over time, adjusted for demographic and clinical risk factors. The outcome measure for glycemic control was HbA(1c). Glycemic control rates were defined as the proportion of type 2 diabetic patients with HbA(1c) level <7%. Dietary treatment in individuals with diabetes decreased as the sole therapy from 27.4 to 20.2% between the surveys. Insulin use also decreased from 24.2 to 16.4%, while those on OADs only increased from 45.4 to 52.5%. Combination of OADs and insulin increased fr! om 3.1 to 11.0%. Glycemic control rates declined from 44.5% in NHANES III (1988-1994) to 35.8% in NHANES 1999-2000. Treatment regimens among U.S. adults diagnosed with type 2 diabetes have changed substantially over the past 10 years. However, a decrease in glycemic control rates was also observed during this time period. This trend may contribute to increased rates of macrovascular and microvascular diabetic complications, which may impact health care costs data support the public health message of implementation of early, aggressive management of diabetes (Koro et al., 2004). Usual insulin, which is: the regular, the neutral protamin Hagedorn and ultralente have 2 characteristics that cause difficulties to the therapy. The first one is that their absorption profile is unpredictable, forming day-to-day changes in the glycemic control

(Lepore et al., 2000). In the study of Lepore et al, the aim of the study was to compare the pharmacokinetics/dynamics of the long-acting insulin analog glargine with NPH, ultralente, and continuous subcutaneous ! (SC) infusion of insulin lispro (continuous subcutaneous insulin infusion [CSII]), 20 C-peptide-negative type 1 diabetic patients were studied on four occasions during an isoglycemic 24-h clamp. Patients received SC injection of either 0.3 U/kg glargine or NPH insulin (random sequence, crossover design). On two subsequent occasions, they received either an SC injection of ultralente (0.3 U/kg) or CSII (0.3 U x kg(-1) x 24 h(-1)) (random sequence, crossover design). After SC insulin injection or CSII, intravenous (IV) insulin was tapered, and glucose was infused to clamp plasma glucose at 130 mg/dl for 24 h. Onset of action (defined as reduction of IV insulin >50%) was earlier with NPH (0.8 +/- 0.2 h), CSII (0.5 +/- 0.1 h), and ultralente (1 +/- 0.2 h) versus glargine (1.5 +/- 0.3 h) ($P < 0.05$) (mean +/- SE). End of action (defined as an increase in plasma glucose >150 mg/dl) occurred later with glargine (22 +/- 4 h) than with NPH (14 +/- 3 h) ($P < 0.05$) but was similar with! ultralente (20 +/- 6 h). NPH and ultralente exhibited a peak concentration and action (at 4.5 +/- 0.5 and 10.1 +/- 1 h, respectively) followed by waning, whereas glargine had no peak but had a flat concentration/action profile mimicking CSII. Interindividual variability (calculated as differences in SD of plasma insulin concentrations and glucose infusion rates in different treatments) was lower with glargine than with NPH and ultralente ($P < 0.05$) but was similar with glargine and CSII (NS). In conclusion, NPH and ultralente are both peak insulin. Duration of action of ultralente is greater, but intersubject variability is also greater than that of NPH. Glargine is a peakless insulin, it lasts nearly 24 h, it has lower intersubject variability than NPH and ultralente, and it

closely mimics CSII, the gold standard of basal insulin replacement (Lepore et al., 2000). The second one is that their activity asks for their management of their serving of food and shot. Insulin therapy can begin at any time and can manage the glycemic control. Intens! ive insulin therapy was discovered that it reduced the chance of aggravating the disease and to reduce the death in people that were very ill in relation to the common therapy (van den Berghe et al., 2001). In the study of van den Berghe et al, it was shown that hyperglycemia and insulin resistance are common in critically ill patients, even if they have not previously had diabetes. In addition, it was also shown that intensive insulin therapy is good to maintain blood glucose at or below 110 mg per deciliter to reduce morbidity and mortality among critically ill patients in the surgical intensive care unit (van den Berghe et al., 2001).

Antioxidant Therapy for Diabetes Complication

It has been proven that a desirable glycemic control is a good way to reduce complications in patients with type 1 diabetes (Bell et al., 2006). In the study of Bell et al, a beta-blocker therapy has been shown to enhance survival in individuals with chronic heart failure although, this class of drugs tends to be underutilized in diabetic patients due to concerns about adverse metabolic effects, especially on glycemic control. No randomized medical examination has particularly estimated the result of beta-blocker therapy on mortality in diabetic patients with heart failure. Previous meta-analyses joining results of heart failure examinations with pharmacologically different beta-blockers indicate that the survival advantage in diabetic patients may be reduced

compared to advantages in non-diabetic patients. Still, some examination results specify that carvedilol, which blocks beta1-, beta2-, and alpha1-receptors and is a potent antioxidant, may create at least similar e! ffects in both patient groups. The objective of this study was to evaluate the effect of carvedilol in patients with heart failure and diabetes, particularly to establish if the survival benefit of carvedilol established in heart failure trials was as great in the subgroups of patients with diabetes. A meta-analysis was completed that included 5757 patients with heart failure, 25% of whom had diabetes, from seven large placebo-controlled randomized examinations with carvedilol. The endpoint of all-cause mortality was investigated in the overall population, patients without diabetes, and patients with diabetes. The number of patients needed to treat for 1 year to prevent one death associated with carvedilol use in diabetic versus non-diabetic patients was also analyzed. The log-rank test and the Cox proportional hazards regression were used to compare the event time distributions of carvedilol versus placebo with respect to the outcome of mortality. Similar survival benefits! were seen with carvedilol use in diabetic and non-diabetic patients. In addition, there were no major differences between the relative mortality risk reductions or the number of patients needed to treat with carvedilol use in diabetic versus non-diabetic patients. The number of patients needed to treat for 1 year to prevent one death was 23 for all patients, as well as for non-diabetic patients, and 25 for the diabetic group. This meta-analysis presents the confirmation that the same survival benefit may occur with carvedilol in heart failure patients with and without diabetes. The low number of patients needed to treat in the severe heart failure trial, Copernicus, and the diabetic subgroup in this meta-analysis indicate that severe heart failure patients and heart failure patients with diabetes may

particularly derive benefit from therapy with carvedilol (Bell et al., 2006). Uncontrolled diabetes got to pronounced oxidative stress that was turned back when patients reached the desired level of glycemic control through a particular remedy wi! th glibenclamide or glicaxide (Chugh et al., 2001). Antioxidant therapy remains a good therapy, but it needs to be explored more. In the study of Chugh et al, parameters of oxidative stress were administrated in 50 patients with type 2 diabetes mellitus in uncontrolled state and after control using oral glibenclamide or gliclazide. The estimates were further compared between the two groups irrespective of drug used to evaluate the difference, if any. The study was a double blind, uncontrolled, noncrossover and randomized trial. Fifty patients of uncontrolled type 2 diabetes were divided in to two groups. Group I (25 patients) received capsule A (glibenclamide) while Group II (25 patients) received capsule B (gliclazide). The parameters studied were Superoxide dismutase (SOD), malonyl-dialdehyde (MDA) and reduced glutathione (GSH). They were done at (a) uncontrolled stage (FBS--165 +/- 16.7 mg/dl, PP--240 +/- 30.1 mg/dl and HbA1--10.5 +/- 0.9% in group I and FBS--150 +/- 15.! 8 mg/dl, PP--246 +/- 29.1 mg/dl HbA1 10.6 +/- 0.8% in group II) and during controlled stage at 12 weeks (FBS--120 +/- 18.5 mg/dl, PP--180 +/- 19.1 mg/dl and HbA1--8.4 +/- 0.29% in group I and FBS--118 +/- 17.6 mg/dl, PP--176 +/- 20.1 mg/dl and HbA1--8.5 +/- 0.39% in group II patients). The significantly raised levels of MDA and SOD, and decreased levels of reduced glutathione (GSH) during uncontrolled stage of diabetes indicated free radical stress induced lipid peroxidation. The significant fall of MDA and SOD and increased levels of GSH in blood in both groups after control revealed beneficial effects of glycemic control on oxidative stress. The levels were not normalized and stayed higher than those in controls. On intergroup

comparison, the control of diabetes with gliclazide (group II) showed improvement in oxidative stress (MDA, GSH) better ($p < 0.001$) than glibenclamide (group I). Oxidative stress in uncontrolled diabetes is decreased with glycemic control. The control of diabetes with gliclazide reduced oxidative stress more than glibe! nclamide, indicating higher antioxidant properties of gliclazide. Normalization of oxidative stress was not achieved. Further studies are required to see long-term effect of drug therapy in combating oxidative stress after achieving acceptable control of diabetes (Chugh et al., 2001).

CONCLUSIONS

Oxidative stress has been demonstrated to participate in the progression of diabetes. Oxidative stress plays an important role during diabetes, including impairment of insulin action, elevation of the complication incidence. Antioxidants have already shown to be prospective in the treatment of diabetes both type 1 diabetes and type 2.

Treatments for diabetes that are specified in this review to prevent against oxidative stress are glutathione, glutathione reductase and peroxidase, vitamins, catalase, superoxide dismutase and antioxidants reserves. The antioxidants that work as inhibitors in the destructive effects of oxidation are needed for improving or stopping the development of diabetes. Physical exercise and insulin therapy that improve the diabetes have also been associated with their antioxidant effects. Further understanding of the mechanisms that relate to the development diabetes will provide more effective therapeutic choices for this devastating disease.

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