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Therapeutic interventions and oxidative stress in diabetes

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1. ABSTRACT

Many therapeutic agents that are used in patients with diabetes mitigate oxidative stress. These agents are of particular interest because oxidative stress is elevated in diabetes and is thought to contribute to vascular dysfunction. Agents that merely quench already formed reactive oxygen species have demonstrated limited success in improving cardiovascular outcomes. Thus, although vitamin E, C, and alpha lipoic acid appeared promising in animal models and initial human studies, subsequent larger trials have failed to demonstrate improvement in cardiovascular outcomes. Drugs that *limit the production of oxidative stress are more successful in improving vascular outcomes* in patients with diabetes. Thus, although statins, ACE inhibitors, ARBs and thiazolinediones are used for varied clinical purposes, their increased efficacy in improving cardiovascular outcomes is likely related to their success in reducing the production of reactive oxygen species at an earlier part of the cascade, thereby more effectively decreasing the oxidative stress burden. In particular, statins and ACE inhibitors/ ARBs appear the most successful at reducing oxidative stress and vascular disease and have potential for synergistic effects.

2. INTRODUCTION

The unifying theory that hyperglycemia induced elevations in superoxide production underlie the activation of many pathways involved in the pathogenesis of diabetic vascular disease naturally raised an interest in the role of antioxidant treatment. However, it appears that not all antioxidants improve vascular function. In fact, antioxidants that simply neutralize the excess oxidative burden present in diabetes appear less effective than antioxidants that block production of reactive oxygen species and limit the cascading increase in oxidative stress. In the following review, we briefly discuss the importance of oxidative stress in mediating vascular dysfunction in diabetes, common surrogate measures of vascular disease and finally, we review the vascular benefits of several therapeutic agents that have known antioxidant properties.

3. DIABETES IS AN OXIDATIVE STRESS DISORDER

3.1. Increased superoxide production

The diabetic state is associated with excess superoxide production. The failure of insulin to stimulate

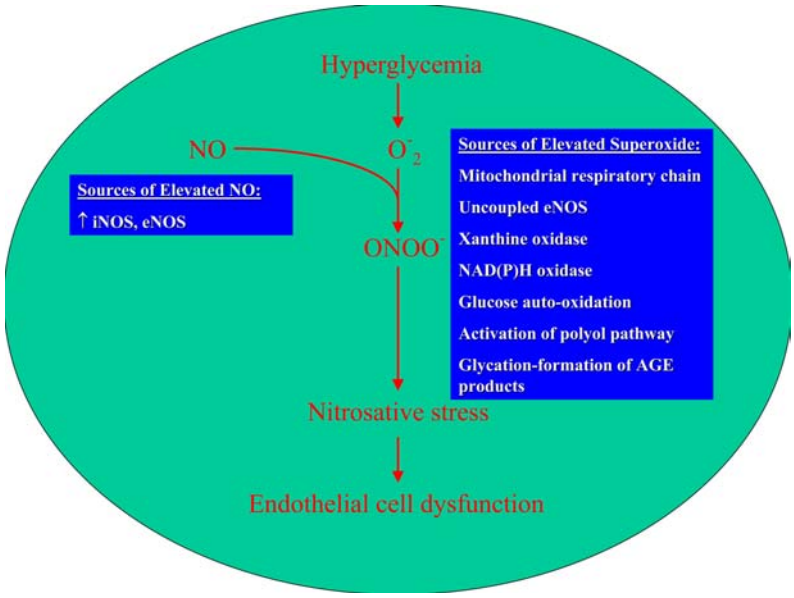

Figure 1. Hyperglycemia induced endothelial dysfunction. Superoxide produced secondary to hyperglycemia combines with NO to form peroxynitrite. This reduces the bioavailability of NO and induces nitrosative stress by multiple mechanisms including modifications of macromolecules and PARP induction.

glucose uptake by fat and muscle tissues, results in hyperglycemia; this causes an increase in intracellular glucose concentrations in insulin-independent cell types, such as endothelium. Increased intracellular glucose concentrations result in an increased rate of glycolysis, which in turn increases the flux of pyruvate (the product of glycolysis) through the tricarboxylic acid (TCA) cycle. It is the increased flux of pyruvate through the TCA cycle that appears responsible for over-production of superoxide. The mitochondrial electron transport chain prematurely transfers electrons to oxygen thereby generating excess superoxide (1). It should be noted that hyperglycemia is not the only mechanism by which diabetes causes increased superoxide production. Diabetes is also associated with increased levels of free fatty acids, which contribute to increased superoxide production (2).

3.2. Oxidative stress and NO

Increased superoxide and reactive oxygen species negatively affect vascular health by downregulating endothelial dervived nitric oxide (NO). NO plays a key role in vasodilation as well as in maintaining a healthy vessel wall by inhibiting inflammation, cellular proliferation and thrombosis. Decreased NO bioavailability not only increases vascular tone, but also promotes structural and biological changes that lead to atherosclerosis. Decreased bioavailability is a result of both NO quenching by peroxynitrite and decreased NO production (1-3)

3.3. Quenching of NO to form peroxynitrite

The superoxide anion reacts with NO to form peroxynitrite, thus reducing the quantity of NO available to the vasculature "see Figure 1". Peroxynitrite post-translationally modifies macromolecules, resulting in impaired protein and lipid function which promotes vascular dysfunction and atherosclerosis (4). The degradation of tyrosine nitrated proteins produces free nitrotyrosine. This marker of nitrosative stress has been found in tissues, atherosclerotic lesions and blood (5, 6, 7). In addition to modification of biomolecules, peroxynitrite may also modulate important signaling pathways and trigger mitochondrial dysfunction and cell death in endothelial cells and cardiomyocytes as described further below (8).

3.4. Decreased NO production

Peroxynitrite also inactivates (6R)-5,6,7,8-tetrahydro-L-biopterin (BH4), a cofactor involved in the production of NO. BH4 oxidation uncouples endothelial NO synthase (eNOS), the enzyme responsible for NO production. Normally, production of NO requires dimerization of eNOS, the presence of L-arginine, and the cofactor BH4. When these conditions are present, eNOS oxidizes its substrate L-arginine to produce L-citrulline and NO. BH4 deficiency uncouples the eNOS complex and promotes production of superoxide by eNOS, thus producing more oxidative stress "see Figure 2".

3.5. Oxidative stress or nitrosative stress and PARP activation

Oxidative stress or nitrosative stress, in the form of peroxynitrite also causes DNA single strand breaks and is one source of poly(ADP-ribose) polymerase (PARP) activation (4). The activation of PARP is an important mediator of vascular dysfunction in diabetes (8-10). PARP activation initiates a series of cell cycle events "see Figure 3" that deplete intracellular nicotinamide adenine dinucleotide (NAD) and adenosine 5'-triphosphate (ATP) pools, thus limiting glycolysis and mitochondrial respiration, leading to vascular cell dysfunction and death (3). Protein kinase C (PKC) activity, advanced glycation

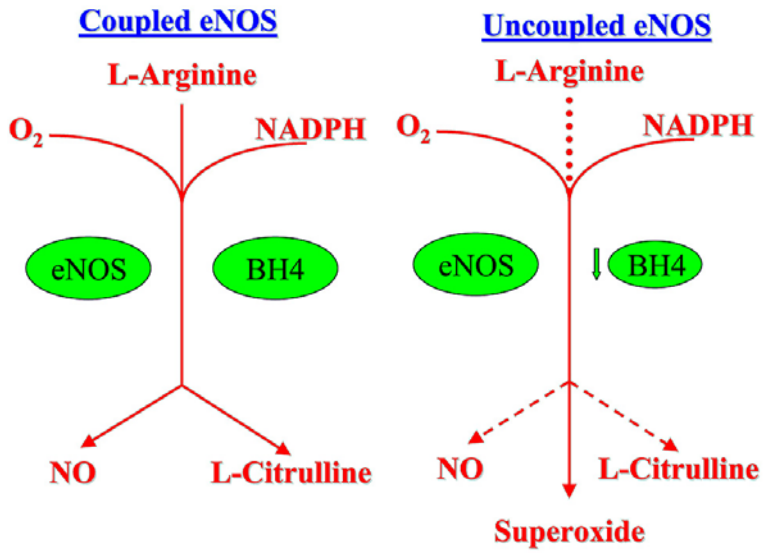

Figure 2. Coupled and uncoupled eNOS. (a) Coupled eNOS utilizes O_2, L-Arginine and NADPH to produce NO and L-Citrulline (b) eNOS can be uncoupled by BH_4 deficiency to produce superoxide rather than NO, which may further reduce available NO by combining with it to form peroxynitrite.

end-product/ receptor for advanced glycation end-product (AGE/RAGE) interactions, and the hexosamine pathway can also be activated by PARP activation as a result of Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) dysfunction (11, 12). PARP activation is elevated in subjects with diabetes and is associated with an impairment in vascular reactivity (13). Recent evidence suggests that increased PARP acitivity is present in subjects with diabetes even prior to the onset of microvascular disease (14).

3.6. Further pathways activated by increased superoxide production

In addition to elevated levels of peroxynitrite, decreased NO and PARP activation, at least four cellular processes have been previously noted to contribute to diabetic microvascular complications. These include: 1) increased flux through the polyol/ aldose pathway, 2) activation of PKC, 3) increased production of AGEs and 4) increased flux through the hexosamine pathway. All of these pathways are activated by hyperglycemia induced superoxide production (15). "see Figure 4"

3.7. PKC activation

Hyperglycemia induced elevations in superoxide anion activate PKC, and activation of PKC further contributes to superoxide generation (1). PKC may also be activated by chronically elevated diacylglycerol (DAG) levels from increased *de novo* synthesis of DAG from glycolytic intermediates, increased activity of the polyol pathway, and via ligation of RAGE (16). The DAG-PKC pathway is activated to maximal levels in three to five days after the initiation of hyperglycemia and remains elevated for many years (17, 18). The activation of PKC increases the activity of membrane associated nicotinamide adenine dinucleotide phosphate (NADPH) oxidases which generate superoxide anion (19). Thus, PKC activation by oxidative stress generates more oxidative stress, creating a vicious circle of positive feedback.

Increased PKC activity is associated with abnormal vascular function and although blocking PKC activity appears to improve microvascular function in animal models, it has little benefit in humans. Activation of PKC results in abnormal vasodilation, increased vascular permeability, increased microvascular protein accumulation, increased plasminogen activator inhibitor-1 (PAI-1) expression, and activation of nuclear factor-kappa B (NF-kB) in endothelial cells and vascular smooth muscle cells. Inhibition of PKC with ruboxistaurin (or LY333531) greatly improves microvascular flow to the retina, kidney, endoneural blood supply and mesenteric bed in animal models (15, 20, 21). Despite these promising findings, ruboxistaurin has had less robust results in humans (22).

3.8. Advanced glycation end products and receptor for advanced glycation end products

AGEs are formed intra- and extracellularly non-enzymatically when reducing sugars combine with free amino groups of proteins, lipids, and guanyl nucleotides. These reactions are irreversible for the most part and accumulate with time. AGEs can alter the structure and function of intra- and extracellular proteins by forming covalent crosslinks. In addition, AGEs help make lipids more atherogenic by glycation and subsequent oxidation. AGEs also cause production of reactive oxygen species and block endothelial NO activity (23).

In addition to their direct effects on macromolecules, AGEs also bind and activate RAGE. Activation of RAGE by AGEs results in sustained activation of NF-kB and its target genes (24). AGE-bound RAGE also increases endothelial cell permeability to macromolecules. Elevated levels of AGEs have been noted

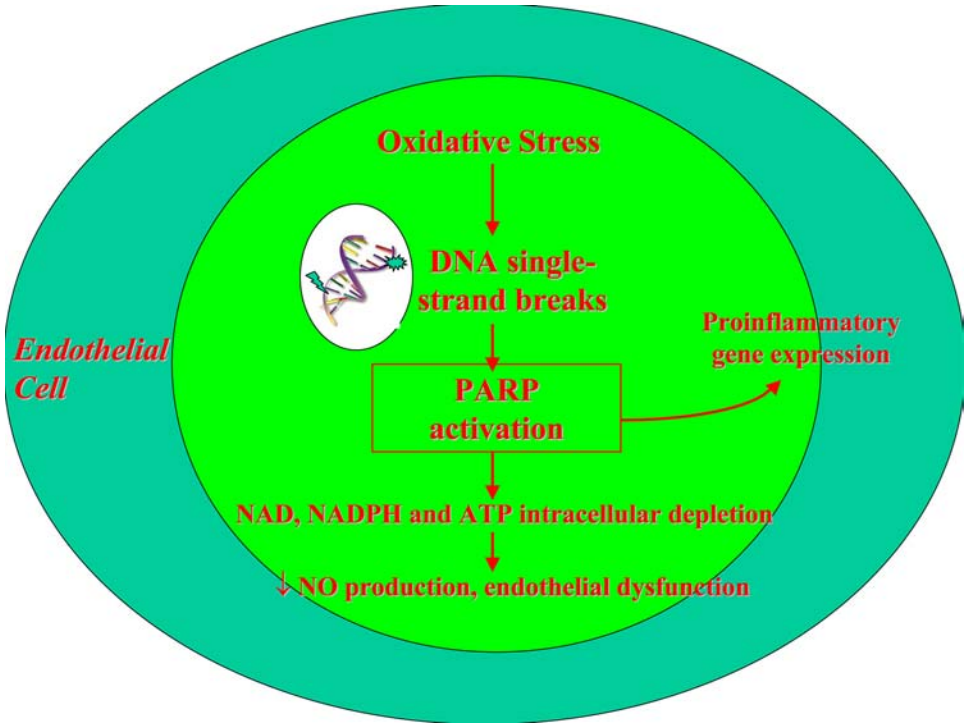

Figure 3. The role of peroxynitrite and PARP. Peroxynitrite induced PARP activation depletes intracellular NAD, NADPH and ATP pools leading to endothelial dysfunction.

in the serum of diabetic patients and correlate with progression of diabetic complications such as nephropathy (25, 26). Treatment of animals with inhibitors of AGE formation, such as aminoguanide, can prevent diabetic microvascular complications (27).

3.9. Polyol pathway

Increased intracellular glucose generates increased flux through the polyol pathway, by engaging the key enzyme, aldose reductase, which usually has a low affinity for glucose. Aldose reductase reduces glucose to sorbital, which is further oxidized to fructose, which consumes cellular NADPH, increasing cellular oxidative stress. Increased flux through the polyol pathway has been implicated in activation of PKC. Inhibition of aldose reductase has been shown to prevent diabetic nephropathy, retinopathy, and neuropathy in animal models (15). Larger clinical trials in humans, however, have had mixed results, thus raising questions regarding the importance of this mechanism (28, 29).

3.10. Hexosamine pathway

Hyperglycemia also shunts glucose through the hexosamine pathway. A glycolytic intermediate, fructose-6-phosphate (Fruc-6P) is converted with glucosamine-6-phosphate, and ultimately to N-acetylglucosamine. Hyperglycemia is associated with an increase in O-linked N-acetylglucosamine modification and decreases O-linked phosphorylation of the transcription factor Sp1, resulting in increased gene expression of transforming growth factor beta (TGF-beta) and PAI-1.(15) Elevated glucose levels also result in inhibition of eNOS, which is accompanied by a twofold increase in O-linked N-acetylglucosamine modification of eNOS and a reciprocal decrease in O-linked serine phosphorylation (30).

4. VASCULAR DISEASE IN DIABETES

Endothelial dysfunction in both the micro- and macro-circulation is the final result of oxidative stress initiated, self perpetuating cascade of events (31). Progressive capillary changes including neovascularizton in retinopathy, and narrowing and/or microthrombosis in peripheral neuropathy are the result of hyperglycemia induced increases in endothelial cell permeability, vascular inflammation, and other structural changes. A reduction in hyperglycemia by intensive glycemic control protocol has been shown in two separate landmark trials to decrease progression and occurrence of microvascular complications (retinopathy, neuropathy, and nephropathy) in *both* type 1 and 2 diabetes (32, 33).

In contrast, glycemic control has been demonstrated to *conclusively* improve macrovascular outcomes in only *type 1 diabetes*. Despite this, macrovascular disease such as myocardial infarction (MI), cerebrovascular accidents, and peripheral arterial disease continues to account for a substantial portion of the mortality and morbidity in both type 1 and 2 diabetes. Improved glycemic control in type 1 diabetes has been associated with dramatically lower rates of macrovascular disease (42% decrease) (34, 35). However, despite reductions in all cause mortality associated with tighter glycemic control macrovascular event rates in type 2

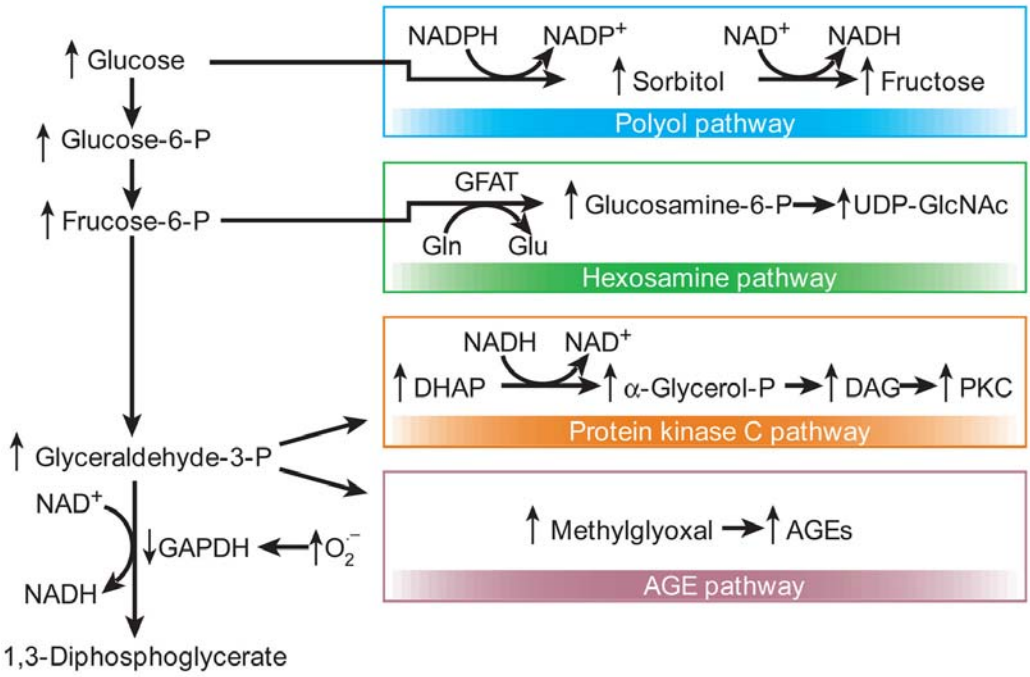

Figure 4. Hyperglycemia induced cellular pathways. Hyperglycemia-induced mitochondrial superoxide overproduction activates the polyol, hexosamine, protein kinase C and AGE pathways. Additionally, excess superoxide production inhibits GAPDH, thus diverting upstream metabolites from glycolysis to the above mentioned pathways. Figure adapted with permission from the publisher (15).

diabetes are not improved with tighter glycemic control unless metformin was part of the regimen (33, 36). Patients with metformin included as part of their regimen are better able to maintain glycemic control over 3 years compared to other regimens and have greater improvements in all cause mortality and decrease in stroke rates (37). Thus, treatment of usual cardiovascular risk factors such as hyperlipidemia and hypertension in type 2 diabetes plays a larger role in lowering the risk of macrovascular events, suggesting that oxidative stress induced by these traditional cardiovascular risk factors appears more important than that induced by hyperglycemia in such patients.

5. METHODS OF ASSESSING ENDOTHELIAL FUNCTION

Prior to the development of macrovascular and microvascular clinical disease early changes in endothelial function can be measured. These changes reflect alterations in the regulation of vascular tone or reactivity which is influenced by endothelial NO production (endothelial-*dependent* vasoreactivity) as well as vascular smooth muscle relaxation in response to NO (endothelial-*independent* vasoreactivity). In endothelial dependent vasodilation, acetylcholine, shear stress or hypoxia can activate endothelial cells to release NO. The stimuli of shear stress and hypoxia are utilized in the flow mediated dilation (FMD) technique to produce endothelium-dependent vasodilation. In contrast, endothelium-independent vasodilation occurs as a result of smooth muscle cell relaxation in direct response to exogenous NO (from NO donors such as nitroglycerin or nitroprusside). Vasoreactivity, which refers to both endothelial dependent and independent vasodilation in response to a stimulus, is a means to quantify endothelial cell and vascular smooth muscle function.

5.1. Macrocirculatory measurements

Macrovascular disease is most commonly assessed by ultrasound measurements of brachial artery diameter and the common carotid intima-media thickness (IMT). Changes in brachial artery diameter after stimuli measure early functional changes associated with atherosclerosis. Endothelium-*dependent* vasodilation of the brachial artery can be assessed by intra-arterial infusion of substances that act on the endothelium to release NO, such as acetylcholine, or by FMD. FMD is induced by occluding the brachial artery with a pneumatic tourniquet to the upper limb for a total of 5 minutes (38). Tissue hypoxia and pH changes in the area distal to the occlusion cause reactive vasodilation in the skin and muscle microcirculation immediately after release of the occlusion. This process causes a brief period of high blood flow and increased shear stress in the brachial artery that stimulates the endothelial production of NO and vasodilation that can be measured on high resolution ultrasound. “see Figure 5” Endothelium-*independent* vasodilatory function of the brachial artery can be assessed by intra-arterial or sublingual administration of NO donors such as nitroglycerin or nitroprusside.

In contrast, common carotid IMT identifies anatomic changes consistent with early atherosclerosis.

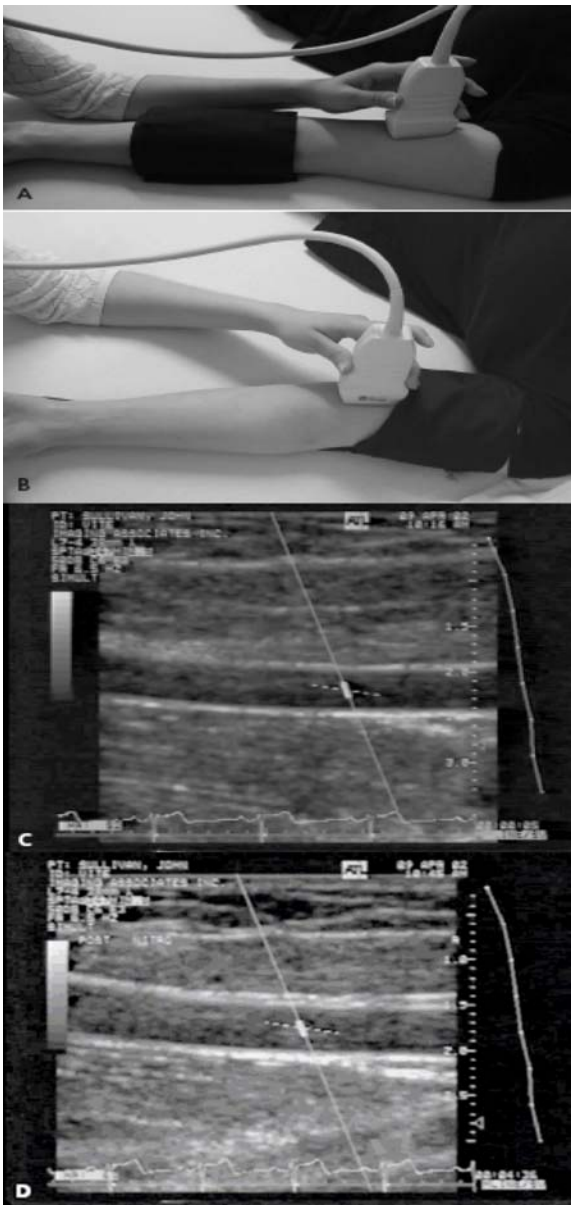

Figure 5. Assessment of FMD in the brachial artery.A 7.0-MHz or greater liner array transducer is used to image the brachial artery above the antecubital fossa in the longitudinal plane. A blood pressure cuff is employed to occlude the arterial blood flow and can be placed either at the forearm (a) or the upper arm level (b). Two-dimensional grayscale scans are taken, one at rest at rest, before the cuff inflation (c) and 1 minute after the cuff deflation that leads to arterial dilation (d). The percentage of the postocclusive artery diameter increase over the baseline represents the FMD. Figure adapted with permission from A. Veves (145).

Carotid artery intima-media thickness (IMT) is an ultrasound measure of the distance between the intima to the outer edge of the media. Increased intima-media thickness occurs early in the process of atherosclerotic plaque formation prior to luminal narrowing. IMT is associated with the presence of conventional atherosclerotic risk factors and can predict the development of cardiovascular events (39, 40).

5.2. Microcirculatory measurements

Microcirculatory vascular reactivity is most commonly assessed by LASER Doppler flowmetry to measure blood flow in the skin. Blood flow is estimated from the combination of number and velocity of moving red cells within arterioles, capillaries, and postcapillary venules. A LASER beam is delivered to the skin via a fiber optic light guide, and reflected light is gathered by a second set of photodetectors. Light reflected by moving objects, such as red blood cells, is reflected at a different frequency. The Doppler shifted fraction of the light signal and the mean Doppler frequency shift is calculated to generate a value in mV, which is proportional to the quantity and velocity of red blood cells with the measured superficial skin microcirculation (41).

The microcirculation can be studied without systemic side effects by using iontophoresis and microdialysis techniques that allow for precise, local delivery of vasoactive agents. Iontophoresis uses a small charge to facilitate transcutaneous delivery of charged substances into the skin without trauma or pain. The length of stimulation, strength of current used, and area of delivery determine the number of molecules transported. Endothelium-dependent vasodilation is assessed by delivery of acetylcholine using anodal current given its positive charge, whereas endothelium-independent vasodilation is assessed by the delivery of the anion sodium nitroprusside using cathodal current. Microdialysis can be used to deliver larger, water-soluble vasoactive agents that lack a charge. These techniques allow for non-invasive measurement of abnormal endothelial function prior to the development of overt clinical disease.

6. THERAPEUTIC INTERVENTIONS THAT MODIFY OXIDATIVE STRESS

In subsequent sections we will discuss how these measurements of vascular function, animal models, and larger clinical trials have been used to evaluate the efficacy of therapeutic agents in combating the increased oxidative stress in diabetes and subsequent ill effects on the vasculature. These agents include vitamins E, C, alpha-lipoic acid, statins, angiotensin converting enzyme inhibitors (ACE inhibitors), angiotensin II receptor blockers (ARBs) and thiazolinedones. Many other agents have been noted to have antioxidant properties, but have not been evaluated in human clinical trials, and are beyond the scope of this review. Therefore, we will limit our discussion to those compounds noted above.

6.1. Vitamin E

Vitamin E is a fat soluble vitamin with known anti-oxidant properties. It is frequently co-administered with vitamin C because its oxidized form is regenerated by vitamin C. Vitamin E and C supplementation improves markers of oxidative stress and endothelium-dependent vasodilation in experimental diabetic models (42-45).

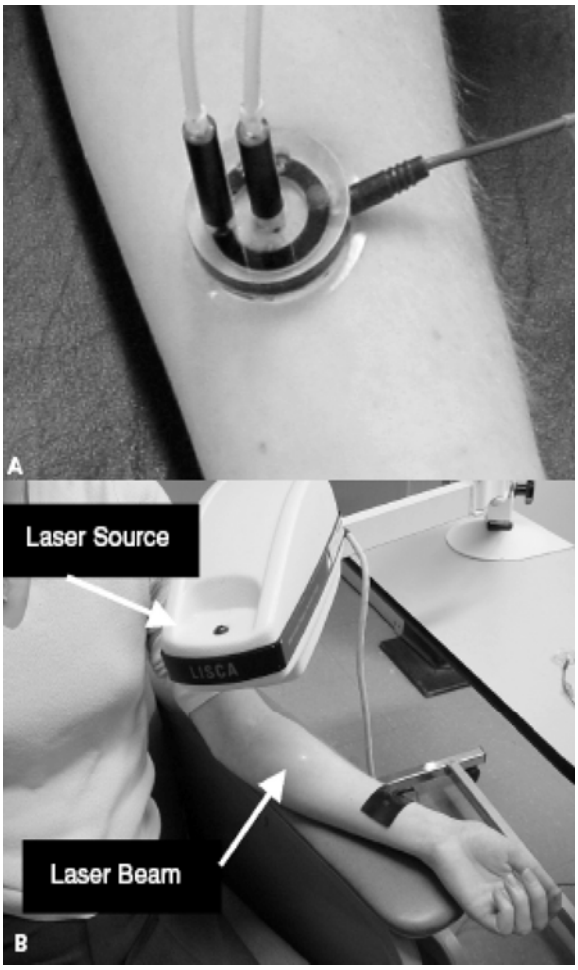

Figure 6. Measurement of skin microcirculation. (a) A small quantity of (<1ml) of 1% Ach chloride solution or 1% sodium nitroprusside solution is placed in the iontophoresis chamber. A constant current of 200 mA is applied for 60 seconds achieving a dose of 6 mC/cm^2 between the iontophoresis chamber and a second nonactive electrode placed 10 to 15 cm proximal to the chamber (black strap around the wrist). This current causes a movement of solution to be delivered toward the skin. (b) Laser Doppler flowmetry: A helium-neon laser beam is emitted from the laser source to sequentially scan the circular hyperemic area produced by the iontophoresed vasoactive substance to a small area on the volar surface of the forearm. Figure adapted with permission from A. Veves (145).

Acute administration of vitamin E has generally been shown to improve endothelial dependent brachial artery vasodilatation in both type 1 and 2 diabetes (46).

Chronic administration of vitamin E was found to be vasoconstrictive in patients with type 1 and 2 diabetes (47). Treatment with twelve months of vitamin E at doses of 1,800 IU per day is associated with no improvement in FMD, a deterioration in endothelial independent vasodilation and a trend towards increased systolic blood pressure. Vitamin E does not change microcirculatory responses to either acetylcholine and nitroprusside or progression of diabetic retinopathy after twelve months (47). Thus, chronic administration of vitamin E appears to worsen endothelial independent vasoreactivity and increase blood pressure in patients with diabetes.

Several prospective interventional trials have found that vitamin E decreases cardiovascular events in the general population and in patients with a high risk of cardiac disease, but without diabetes. In particular, vitamin E was reported to reduce the risk of non-fatal MI when administered at doses of 400-800 IU per day in patients with prior symptomatic coronary atherosclerosis (CHAOS trial) (48). However, it is also associated with a disturbing, non-significant trend towards an excess of cardiovascular deaths.

A lack of benefit with vitamin E supplementation in a subsequent series of randomized trials dampened enthusiasm for vitamin E's utility as an antioxidant in diabetes. In fact, both cardiovascular outcomes and atherosclerosis progression by carotid IMT are not improved by vitamin E in a group of high risk patients with vascular disease or diabetes (HOPE study and SECURE trial) (49-52). The lack of benefit in regards to cardiovascular outcomes and nephropathy persisted after a subgroup analysis of patients with diabetes. In addition, there was no reduction in cardiovascular events or death in 1031 patients with diabetes after vitamin E supplementation in the PPP trial (53). In addition, an increased risk of adverse events with vitamin E supplementation raises further concerns about its use. Vitamin E supplementation appeared safe during the initial HOPE study with a follow-up period of 4.5 years, but extended followup for a total of 7 years was associated with an increased risk of heart failure. The excess heart failure risk was also evident after 3.5 years of follow-up of post-infarction patients supplemented with vitamin E in the GISSI-Prevenzione trial (54, 55). In fact, patients with left ventricular dysfunction (ejection fraction <50%) who are treated with vitamin E demonstratd a 50% increased risk of developing congestive heart failure (p=0.034).

In addition, higher doses are associated with increased mortality risk. In a meta-analysis of vitamin E trials prior to August 2004, low dose vitamin E (<150 IU per day) was associated with a non-significant decrease in all cause mortality (56). Doses larger that 150 IU per day are associated with a progressive increase in all cause mortality as dose increased. Furthermore, the benefical effect of low dose vitamin E was attenuated when adjustments were made for concomitant use of other vitamin supplements. Thus, the routine use of vitamin E supplementation with or without vitamin C cannot be recommended in patients with diabetes.

6.2. Vitamin C

Vitamin C, also called ascorbic acid, is a water soluble vitamin with many biological roles in addition to its function as an antioxidant. Vitamin C stabilizes eNOS cofactor BH4, leading to increases in NO production. It also prevents oxidation of LDL and regenerates oxidized

vitamin E. Initial physiologic studies demonstrate improvement in endothelial function with acute infusion of vitamin C in patients with acute hyperglycemia, type I and II diabetes and hypertension (57-60). Longer term, orally delivered vitamin C has variable effectiveness in improving brachial artery reactivity in patients with type II diabetes (61, 62).

Epidemiologic data suggest that higher intake of vitamin C is associated with improvements in mortality, particularly from cardiovascular causes. The First National Health and Nutrition Examination Survey (NHANES I) studied a cohort of 11,348 adults for 10 years (63). In this cohort, increased vitamin C intake (approx 300mg per day) was associated with a 45 to 25% risk reduction in all cause mortality and mortality from cardiovascular causes in men and women respectively. Vitamin C supplement use was associated with a significantly lower risk (28%) of coronary disease (relative risk of 0.72) after controlling for other cardiovascular risk factors in an observational study of 85,118 female nurses followed for 16 years (4, 64). This benefit was noted again by researchers in the EPIC-Norfolk prospective population study (65). The highest quartile of ascorbic acid had an odds ratio for future coronary artery disease of 0.67 compared with those in the lowest quartile. There are no randomized, controlled studies addressing the cardiovascular benefits of vitamin C supplementation independent of other vitamin supplements. Therefore, at this time, the use of vitamin C for cardiovascular benefits cannot be recommended in diabetes or the general population.

6.3. Alpha-lipoic acid

Alpha-lipoic acid is a more potent antioxidant than either vitamin E or C, and a critical cofactor in aerobic metabolism. Alpha-Lipoic acid reduced to its conjugate base, dihydrolipoate, is able to regenerate other antioxidants such as vitamins E and C, as well as reduced glutathione. Thus, one might expect more potent vascular benefits.

Diabetic animal models demonstrate improvements in metabolic profile and the microvasculature after treatment with alpha-lipoic acid. Thus, blood glucose, plasma insulin, cholesterol, triglycerides and lipid peroxidation improvements are associated with increased antioxidant enzymatic activity (catalase and glutathione peroxidase activity) (66). These benefits are partly attributable to the recovery of insulin producing cells in the pancreas, and are significant enough to prevent atherosclerotic lesions (67). In the microvasculature of diabetic rats, alpha-lipoic acid reduces nitrotyrosine levels and prevents pathologic retinal vessel changes (68). In endothelial cell cultures, alpha-lipoic acid prevents AGE dependent depletion of reduced glutathione and ascorbic acid and subsequent activation of NF-kappa B (69). Thus, it appears alpha-lipoic acid supplementation reduces oxidative stress and thereby improves metabolic derangements and microvascular function in animal and *in vitro* models.

Alpha-lipoic acid has been mainly studied in randomized controlled human clinical trials for the treatment of diabetic polyneuropathy. Short term IV alpha-lipoic acid for 19 days appeared to improve symptoms, and longer term alpha-lipoic acid (IV infusions, followed by oral therapy for 2 years) were reported to improve objective peripheral nerve function (70, 71). Prolonged treatment of four years duration in the NATHAN 1 trial found improvements in only some neuropathic deficits and symptoms, but not objective nerve conduction in patients with mild to moderate distal symmetric neuropathy (72). In addition, there was a nonsignificant trend towards an increased rate of serious adverse events from 28% to 38% in the active treatment group. Thus, although there may be a possibility of improvements in neuropathy with short term IV infusion of alpha-lipoic acid, these improvements have not been sustained with long term oral therapy and are may increase the risk of serious adverse events.

The effects of alpha-lipoic acid on autonomic dysfunction and surrogate markers of macrovascular disease have been studied in only small numbers of patients. Alpha-lipoic acid treatment for four months slightly improves measures of heart rate variability, a measure of autonomic dysfunction, but does not change symptoms of autonomic dysfunction (73). Four weeks of therapy with oral alpha-lipoic acid improves endothelium-dependent vasorelaxation of the brachial artery by 44% compared to the placebo group and was accompanied by reductions in markers of endothelial activation, plasma interleukin-6 and plasminogen activator-1 (74). Thus, the impact of lipoic acid on clinical cardiovascular end-points is still unknown. Given this, and the increased risk of serious adverse events with long term use, the use of alpha-lipoic acid supplements cannot be recommended for patients with diabetes.

6.4. Statins

Statins inhibit the enzyme hydroxymethylglutaryl coenzyme A reductase (HMG CoA reductase) thereby improving serum lipid profile and lowering cardiovascular morbidity and mortality (75). These agents were initially thought to exert their beneficial effects on endothelial function secondary to their lipid lowering capacity. However, it appears that improvements in vascular function are only partly mediated by this mechanism (76). Statins also improve endothelial function by decreasing oxidative stress, inflammation and the thrombogenic response (77).

Statins achieve this enhanced vascular function by decreasing NADPH activity, reducing formation of reactive oxygen species and downregulating the renin angiotensin system. Statins reduce activity of the NADPH oxidase in endothelial cells, thus reducing the formation of ROS as well as oxidation of LDL (78-85). In addition, statins decrease uptake of oxidized LDL by monocytes that develop into foam cells in atherosclerotic lesions (86, 87). Atorvastatin is unique in that its hydroxymetabolites are present at usual doses and demonstrate free radical scavenging abilities (88). Statins downregulate AT1 receptor at the transcriptional level further improving measures of oxidative stress and vascular function (81).

All of the above mechanisms upregulate eNOS activity, which plays a central role in mediating the beneficial effects of statins in endothelial cells. Transcription of eNOS is reduced by the presence of oxidized LDL, but not native LDL. Statins prevent this inhibition of eNOS at the transcriptional level (89). This increased expression of eNOS is associated with an improvement in vascular function in animal models of type II diabetes and hypercholesteremia (90, 91). Statin mediated increases in eNOS function appear critical in vascular regeneration and restored myocardial vasorelaxation after experimentally induced myocardial infarction in the mouse model, as these benefits were not observed in eNOS -/- mice after statin treatment (92).

Statins unequivocally reduce the risk of major vascular events such as stroke, myocardial infarction, and coronary revascularization in patients with diabetes (93, 94). However, surrogate markers of such macrovascular events, endothelial dependent vasorelaxation, are not clearly improved with statins. In particular, vasoreactivity does not improve after statin treatment in patients with poorly controlled diabetes (95). Endothelial dependent vasodilation *does* improve independently of lipid lowering *in patients with better glycemic and lipid control* in both type 1 & type 2 diabetes (96-101). Statin use was also reported to ameliorate postprandial hypertriglyceridemic- and hyperglycemia-induced endothelial dysfunction and reduced serum nitrotyrosine levels in type II diabetes suggesting that its short term, lipid-independent vascular benefits are secondary to decreased oxidative and nitrosative stress (102).

Thus, statins improve endothelial function prior to reductions in LDL unless there is overwhelming oxidative stress related to factors such as hyperglycemia and hypercholesteremia in type 2 diabetes. A lack of response to statins may be related to elevated asymmetric dimethylarginine (ADMA) levels, a competitive inhibitor of eNOS. ADMA is elevated in by many cardiovascular risk factors and patients with elevated ADMA levels are less likely to have an improvement in vasoreactivity with statin use after 3 weeks (103).

6.5. ACE-inhibitors and Angiotensin II receptor blockers

Both ACE inhibitors and ARBs exert their clinical effects by decreasing the binding of angiotensin II to the AT1 receptor, by decreasing levels of angiotensin II and by inhibiting the interaction of angiotensin II to the AT1 receptor, respectively. It should be noted that ACE inhibitors reduce formation of angiotensin II by inhibiting ACE1, but have no effect on ACE2 or other angiotensin II forming enzymes. Angiotensin II opposes many of the actions of NO; it causes vasoconstriction, altered vascular smooth muscle function, increased inflammation via NF-kB and hypercoagulability by increased formation of PAI-1. In addition, inflammation itself may sustain endothelial dysfunction by activating the renin-angiotensin system, and subsequently increasing ROS formation and decreasing endothelial dependent vasoreactivity (104). Angiotensin II also induces vascular superoxide production by uncoupling eNOS upon loss of dihydrofolate reductase (DHFR), a BH4 salvage enzyme (105). Thus, ARBs and ACE inhibitors improve endothelium-dependent vasorelaxation by decreasing superoxide production, and increases NO bioavailability (105-108).

ACE inhibitors and ARBs improve vascular function and cardiovascular outcomes in type 2 diabetes. Both agents unequivocally improve endothelial function in patients with type 2 diabetes (109-112). Valsartan therapy improved resting forearm skin blood flow and resting brachial artery diameter after 12 weeks in patients with type 2 diabetes. However, their impact on endothelial function in patients with type 1 diabetes is less clear (113-116). ACE inhibitors and ARBs improve cardiovascular and all-cause mortality outcomes in patients with diabetes, in fact, to a greater degree than in non-diabetics as noted in subgroup analysis of the HOPE and LIFE studies (approx 38% and 19%, respectively, of subjects had diabetes) (117, 118). In addition, both of these agents appear to reduce the onset of type 2 diabetes in susceptible populations. Thus, it appears that ACE inhibitors and ARBs improve vascular outcomes in patients with diabetes.

As briefly mentioned earlier, LDL and the renin-angiotensin system modulate one another. The presence of native LDL increases AT1 receptor expression at least two fold in a sustained manner for 24 hours by stabilization of post-transcriptional mRNA (119). Increased AT1 receptor expression and activity is associated with increased production of superoxide and decreased endothelial-dependent vasodilatation (120). However, statins reduce the half life of AT1 receptor mRNA and thereby reduce angiotensin II induced production of reactive oxygen species (121). Native LDL, not oxidized LDL, increases angiotensin II-AT1 receptor induced vasoconstriction (122-124).

Conversely, angiotensin II binding of the AT1 receptor upregulates endothelial oxidized LDL receptor (LOX-1) in endothelial cells. This upregulation of LOX-1 receptor is prevented by ARBs and ACE inhibitors, thus limiting the potential diffusion of oxidized LDL from the blood into the vessel wall where it can result in plaque formation (125). Thus, co-administration of ACE-inhibitors/ ARBs with a statin may decrease oxidative stress, vasoconstriction, decrease uptake of oxidized LDL and improve endothelial function.

6.6. Thiazolinediones

Thiazolinedones, also known as PPAR gamma agonists, include pioglitazone (Actos), rosiglitazone (Avandia), and troglitazone (Rezulin). These agonists bind nuclear PPAR-gamma receptors in adipocytes that function as transcription factors for genes important in adipocyte differentiation, lipid metabolism and insulin sensitivity. In addition, PPAR-gamma receptors are expressed in cells integral to the development of atherosclerosis: endothelial cells, vascular smooth muscle cells, monocytes/ macrophages and T cells. Thus, one would expect all thiazolinedones to improve vascular function in a similar manner, just as they all improve insulin sensitivity. Indeed,

all thiazolinediones have been demonstrated to enhance glycemic control, and improve surrogate measures of vascular disease. Thiazolinediones improve endothelial dependent vasoreactivity and measurements of carotid IMT in patients with diabetes (126-130). In addition, both rosiglitazone and pioglitazone have been reported to increase the regenerative capacity of endothelial progenitor cells in individuals with diabetes (131, 132). This improvement in vascular function is associated with reduced NADPH oxidase activity, decreased LDL oxidation and reduction in vascular inflammation (130, 133).

Despite these improvements in oxidative stress and vascular function, individual thiazolinediones appear to worsen *clinical* cardiovascular *outcomes*. Cardiovascular outcome data on troglitazone is limited because it was withdrawn from the market shortly after reports of severe, idiosyncratic, often fatal hepatic failure (134). However, rosiglitazone and pioglitazone have been reported to differentially affect the risk of myocardial infarction and both appear to increase the risk of heart failure.

Rosiglitazone was reported to elevate the risk of myocardial infarction and congestive heart failure. A meta-analysis by Nissen and Wolski reported a 43% increased risk of myocardial infarction (MI) and increased risk of death from cardiovascular causes (by 64%) with rosiglitazone therapy (135). These results were also supported by a meta-analysis of data from the manufacturer, GlaxoSmithKine, which reported a 31% increased risk of MI (136). In response to this data, the RECORD trial (which was sponsored by the manufacturer) performed an unplanned interim analysis which revealed no increased risk of MI or death from cardiovascular causes with rosiglitazone treatment in patients with type 2 diabetes (137). However, at the time of the interim analysis, the study lacked power to substantiate this negative finding. Recently, another new meta-analysis of long term (>12 months), randomized controlled trials in subjects that had impaired glucose tolerance or diabetes was performed because the Nissen and Wolski meta-analyiss included many small studies of short duration with heterogenous populations. This report again confirmed a similar increased risk of myocardial infarction (42% increase), but without the increase in cardiovascular mortality (138). Thus, the current consensus is that rosiglitazone may have detrimental effects in patients with previous heart disease and diabetes, and its use cannot be recommended in these patients.

Rosiglitazone has also been associated with an increased risk of congestive heart failure. In particular, rosiglitazone treatment was associated with an increased risk of heart failure (hazard ratio 2.15) in the interim analysis of the RECORD trial as well as in the most recent meta-analysis by Singh et. al (RR 2.09) (137, 138). It appears that this increased risk of heart failure can be mitigated by close attention to fluid status. Thus, rates of heart failure or worsening heart failure were not elevated in a group of patients with diabetes and mild heart failure (139). However, the rosiglitazone treated group suffered from significantly more cases of worsening edema (25.5% vs. 8.8%, P = 0.005), an increase in heart failure medication (33% vs. 18%), as well as, small, statistically significant, increases in brain natriutetic peptide levels. Despite the increased edema formation associated with rosiglitazone treatment, it was not reported to cause structural changes in left ventricular size or function in patients without a prior history of heart failure (140). Thus, rosiglitazone appears to worsen heart failure and its use cannot be recommended in patients with heart failure or those at risk of heart failure.

Unlike the first two thiazolinediones, larger clinical trials of pioglitzone in high-risk patients with type 2 diabetes and prior MI demonstrate an improvement in rates of myocardial infarction, but increased edema formation and heart failure remain concerns. After a median follow up of almost 3 years, pioglitazone was reported to reduce the risk of MI by 28%, acute coronary syndromes (ACS) by 37%, and the composite end point of nonfatal MI, coronary revascularization, ACS, and cardiac death by 19% (141). In addition, pioglitazone therapy has been reported to improve nocturnal blood pressure (142). The above findings were confirmed by a recent meta-analysis by Nissen et al which reported a slight decrease in the composite end point of death, myocardial infarctio, or stroke (hazard ration 0.82) and a slight increase in the risk of serious heart failure (hazard ratio 1.41) after approximately a year of pioglitazone therapy (143). Much of the differential benefit of pioglitazone is likely due to its favorable lipid profile. It improves total cholesterol, LDL, HDL and triglycerides, whereas rosiglitazone therapy increases in total cholesterol, LDL, and triglycerides (144).

7. SUMMARY

In summary, diabetes is a state associated with increased oxidative stress secondary to hyperglycemia and increased free fatty acid production. Agents such as vitamin E, C, and alpha lipoic acid that mitigate this oxidative stress by quenching already formed reactive oxygen species have had limited success in improving vascular function. Drugs which limit the production of superoxide and other reactive oxygen species such as statins, ACE inhibitors, ARBs and thiazolinediones are more successful in improving vascular outcomes in patients with diabetes. Their success is based on limiting the cascade of antioxidant production and subsequent vascular inflammation. In particular, the coadministration of statins and ACE inhibitors/ ARBs will may lead to synergistic reductions in oxidant burden and vascular disease. Additionally, outcome studies are needed to confirm vascular benefits, as measurements of surrogate markers often do not predict longterm clinical outcomes.

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Abbreviations: TCA cycle: tricarboxylic acid cycle; NO: nitric oxide; eNOS: endothelial NO synthase; BH4: (6R)-5,6,7,8-tetrahydro-L-biopterin; PARP: poly(ADP-ribose) polymerase; PKC: protein kinase C; AGE: advanced glycation end products; RAGE: receptor for advanced glycation end products; FMD: flow-mediated dilation; IMT intima-media thickness; ACE inhibitors: angiotensin converting enzyme inhibitors; ARBs: angiotensin II receptor blockers; LDL: low density lipoprotein; HDL: high density lipoprotein; ROS: reactive oxygen species; AT1 receptor: angiotensin II type 1 receptor; MI: myocardial infarction; ACS: acute coronary syndromes

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