

Intertwined role of reactive oxygen, nitrogen and carbon in diabetic state

Abstract

Diabetes is a metabolic disorder of multiple etiologies, characterized by chronic hyperglycemia caused by defects in the secretion of insulin or the action of insulin, or both. Production of reactive oxygen species (ROS) and lipid peroxidation are increased in diabetic patients, especially in those with poor glycemic control. An increase in steady-state level of reactive oxygen species (ROS) or reactive carbonyl species (RCS) may result in so-called “oxidative stress” or “carbonyl stress”, respectively. Reactive nitrogen species (RNS) is also a subset of ROS. ROS and RNS include hydroxyl radical ($\cdot\text{OH}$), hydrogen peroxide (H_2O_2), superoxide ($\text{O}_2\cdot^-$), nitric oxide ($\text{NO}\cdot$), peroxynitrite ($\text{ONOO}\cdot$). In physiological conditions, mitochondria are the major site of intracellular ROS production, due to electron leakage along the respiratory chain. Protein kinase C also becomes activated in retinal endothelial cells under hyperglycemic conditions and can lead to activation of NADH oxidase. Metal-catalyzed auto oxidation of reducing carbohydrates could be involved in the formation of AGEs and ROS. Hexosamine biosynthesis pathway is an additional pathway of glucose metabolism that may mediate some of the toxic effects of glucose. Oxidized lipoproteins activate inflammatory signaling, which in turn aggravates the inflammation. Therefore, clearance of oxidative and nitrosative stress and inhibition of inflammation have been considered good strategies for the treatment of diabetic complications.

Keywords: reactive metabolites, pkc, advanced glycation end products, oxidized ldl, ros

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Abbreviations: ROS, reactive oxygen species; RNS, reactive nitrogen species; RCS, reactive carbonyl species; DAG, diacylglycerol; PKC, protein kinase c; TNF, tumor necrosis factor- α ; IL-6, interleukin-6; RAGE, receptor for advanced glycation end products; ICAM-1, intercellular adhesion molecule-1; UDPGLCNAC, uridine diphosphate-n-acetyl glucosamine; TGF β 1, transforming growth factor β 1; PAI-1, plasminogen activator inhibitor-1; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; Ox-LDL, oxidized ldl; XO, xanthine oxidase(XO); MDA, malondialdehyde; GSH, glutathione; GSSG, oxidised glutathione; ETC, electron transport chain; HMP, hexose monophosphate shunt; NAD, nicotinamide adenine dinucleotide; Gly-Ages, glycated advanced glycation end products; GLAP, glyceraldehyde-derived pyridinium; MCP-1, monocyte chemoattractant protein-1; HUVECS, human umbilical vein endothelial cells; RM, reactive metabolites

Introduction

Diabetes often referred to as an epidemic is one of the major human diseases.¹ According to a report from International Diabetes federation, 387million individuals are diagnosed with diabetes in 2014 which is expected to increase to more than 205million in 2035.² The death due to diabetes in 2014 was 4.9million where the rate is 1 person per every 7seconds. The expenditure for diabetes reached 612 US \$ in 2014. This syndrome is characterized by an elevation of blood glucose, alters the responsiveness of small blood vessels to physiological stimuli, precipitating hypertension and other microvascular related diseases including atherosclerosis, neuropathy and retinopathy.³

Diabetic neuropathy is a serious consequence of long term intracellular glucose metabolism that leads to neuronal damage, resulting in neuronal complications of diabetes.⁴ Elevated blood

glucose levels during diabetic state is a major factor for neuronal damage which involves many metabolic pathways; sorbitol induced formation of reactive oxygen species, advanced glycation end products, Protein Kinase C pathway and Hexosamine pathway.⁵ Cell responds to high glucose by the production of reactive oxygen and nitrogen species.⁶ Nitric oxide is an important vascular target for ROS. Superoxide neutralizes NO and the peroxide formed is a source of hydroxyl radicals that can cause endothelial damage.⁷ Apart from ROS and RNS, diabetes is also characterized by the elevated levels of reactive carbonyl compounds, a phenomenon known as carbonyl stress.⁸ RCS react with both arginine and lysine with high reaction rates which differentiates it from glucose which alters lysine residues specifically.⁹ Among the various RCS species, although the reactivity of 3-deoxyglucose towards nucleophilic groups is considerably lower compared to methylglyoxal and glyoxal, it can modify protein lysine and arginine residues.¹⁰ Auto oxidation of glucose with concomitant formation of glycation end products increases the spontaneous production of reactive species which causes disturbance to the nerve function in diabetic neuropathy.¹¹

Mitochondria as a source of reactive species

Increased flux through the respiratory chain in mitochondria due to hyperglycemic conditions increases the polarity of inner membrane of mitochondria which stimulates formation of ROS in Electron transport chain.¹² $\cdot\text{O}_2^-$ is the initial free radical to be formed which is followed by a series of reactive species that is responsible for cell damage.¹³ In addition to producing ROS, the mitochondrial respiratory chain is capable of producing nitric oxide ($\text{NO}\cdot$).¹⁴ O_2^- can interact with $\text{NO}\cdot$ to form peroxynitrite ($\text{ONOO}\cdot$), a strong oxidant. This and other oxidants derived from $\text{NO}\cdot$ are referred to as reactive nitrogen species (RNS), which oxidizes proteins and nucleic acids.¹⁵ Excessive accumulation of ROS or RNS and their uncontrolled oxidation of

cellular components are referred to as oxidative stress. Excess RNS also affects the nitrosation or nitration of cellular targets, including proteins and glutathione known as nitrosative stress. 4-Hydroxynonenal is a major inducer of oxidative stress and has been associated with a variety of pathophysiological states.¹⁶

Respiratory chain as a source of reactive species

In oxidative phosphorylation, electrons are transferred from glucose through glycolytic pathway, TCA cycle, along a series of complex I to IV and ATP synthase to molecular oxygen. The mitochondrial membrane potential generated due to proton transfer drives the formation of ATP. The series of reactions is tightly regulated. During chronic hyperglycemia, excess substrate feeding into ETC, collapses the proton gradient to form superoxide radicals instead of ATP. The leakage of electrons occurs at complex I and between Coenzyme Q and complex III.¹⁷ What is the main link between increased glucose and main pathways responsible for hyperglycemic damage through ROS production via the mitochondrial respiratory chain? The prevailing hypothesis is that hyperglycemia-induced increase in electron transfer donors (NADH and FADH₂) increases electron flux through the mitochondrial electron transport chain. Consequently, there is an increase of the ATP/ADP ratio and hyperpolarization of the mitochondrial membrane potential. Mitochondria are considered to be the important site for production of ROS within the cell, as electron leaks out from the electron transport chain; though it also arises from plasma membranes, lysosomes and cytosolic action of enzymes. When the concentrations of ROS/RNS is less it induces a range of biological reactions, including signaling reactions and immune mediated action against microbial pathogens; Increased levels of superoxide and peroxy-nitrite radicals damages the lipids, proteins and nucleic acids, which causes inflammation and apoptosis. The electron

transport chain complexes are subjected to fine changes that alters the rate of electron flow. These modifications include allosteric regulation by ATP, nitration of tyrosine of polypeptides of ETC complexes.¹⁸ Synthesis of NO[•] in mitochondria from respiratory chain has been explained in mouse brain mitochondria, human endothelial cells and other organisms. This reduction of NO₂⁻ to NO[•] is stimulated during hypoxia or anoxia and is independent of oxygen.

Reactive metabolites derived due to activation of Protein-kinase C and NADPH oxidase (Figure 1)

PKC activation stimulates NADPH oxidase via a PI-3 kinase-dependent and independent pathway to induce ROS production; and PKC activity is inhibited by cilostazol at the PI-3 kinase level to suppress activation of NADPH oxidase activation in the presence of TNF- α [19]. According to a report by Satoh et al.²⁰ the two important sources of superoxide in glomerular cells are NADPH oxidase and uncoupling of NOS and maintaining normal levels of tetrahydrobiopterin attenuated ROS formation and improved kidney function.²⁰ In spite of its presence in phagocytic cells, NAD(P)H oxidase is also present in nonphagocytes like mesangial and proximal tubular cells, endothelial cells, and fibroblasts.²¹ Reactive species produced within the cells act as second messengers. NADPH oxidase is activated by binding of cytokines, TNF- α , PDGF, angiotensin II to their cognate receptors. In the diabetic condition, the brain tissue is subjected to a varied oxidative state that is followed by an elevated activity of Xanthine oxidase activity (XO, an enzyme involved in the production of reactive oxygen and nitrogen species, ROS and RNS, respectively) as well as in nitric oxide (NO, marker of vascular disorder) and malondialdehyde (MDA, index of polyunsaturated fatty acid oxidation) levels with concomitant decrease in the antioxidants, vitamin C and glutathione (GSH).²²

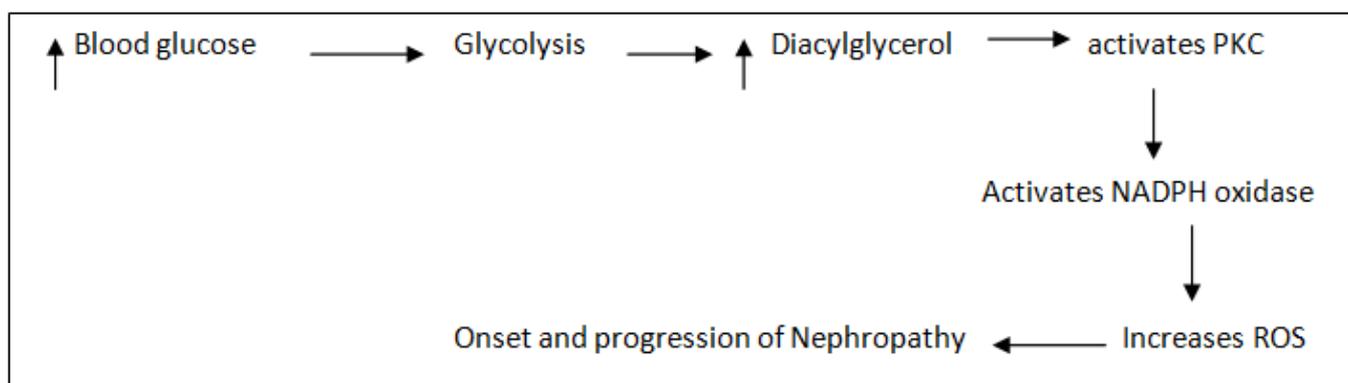


Figure 1 Activation of PKC and NADPH oxidase.

Reactive metabolites from advanced glycation end products

When glucose reacts with amino acids on proteins, AGE formation is initiated by forming a compound which then stimulates further chemical reactions. Elevated levels of AGE are formed under hyperglycemic conditions. Protein glycation modifies cellular function, and AGE binding to their receptors can lead to alteration in cell signaling and formation of free radicals.²³ Studies have shown that AGEs which are highly specific bound to RAGE.²⁴ Interaction between AGE-RAGE stimulates NADPH oxidase and inflammatory response. RAGE- antibody inhibits expression of cytokines induced

by AGE like IL-6, MCP-1, TGF- β 1, ICAM-1 and RAGE. The AGEs are finally removed from kidney. AGEs also bind to many sites at renal cells which stimulates biological effects, of which the preferential member of binding is Immunoglobulin super family.²⁵

Reactive metabolites from Sorbitol pathway (Figure 2)

Glucose flux through polyol pathway leads to NADPH depletion. NADPH is an important cofactor necessary to regenerate reduced glutathione. Depletion of GSH could exacerbate intracellular oxidative stress and thereby contribute to diabetic complications.²⁶ Inhibition of polyol pathway is a potential treatment of diabetic neuropathy.

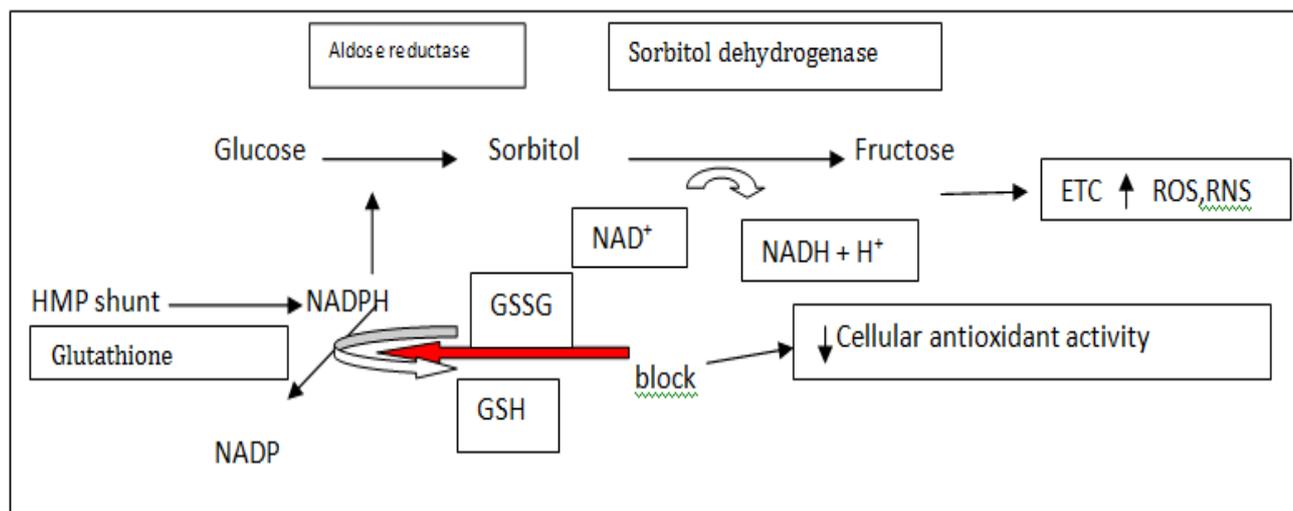


Figure 2 ROS generation through Sorbitol pathway.

Glucose flux through the polyol pathway leads to depletion of NADPH as shown in Figure 1. NADPH is an important cofactor to produce reduced glutathione, (GSH) and GSH is a scavenger of reactive oxygen species (ROS). Depletion of GSH could exacerbate intracellular oxidative stress and thereby contribute to diabetic complications.²⁶

Reactive metabolites from Hexosamine pathway (Figure 3)

Mitochondrial ROS induced by hyperglycemia inhibited the activity of GAPDH. It was also supported by the fact that the downstream

end products of glucose were decreased by H₂O₂. The reason why the upstream metabolites of glucose were not increased is not clear at present. However one possibility is that upstream metabolites could flow into Hexosamine pathway because it was reported that hyperglycemia induced 2.4-fold increase in Hexosamine pathway activity via increased production of mitochondrial superoxide.²⁷

An excess glucose condition creates additional flow through the Hexosamine pathway, resulting in excess formation of GlcNAc and varied levels of gene expression.²⁸ Vascular smooth muscle cell mitosis is promoted due to increased expression of plasminogen activator inhibitor-1, which is involved in atherosclerosis.²⁹

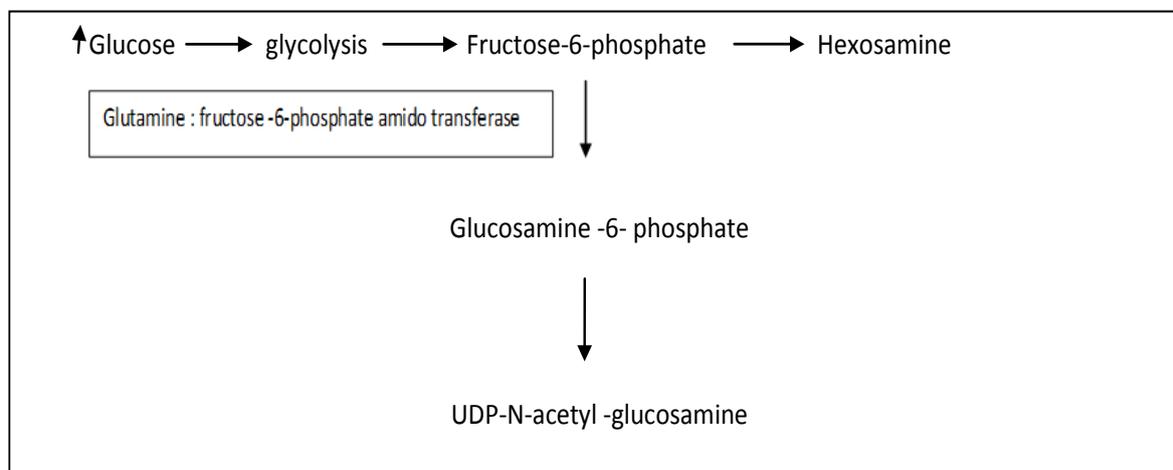


Figure 3 ROS generation through Hexosamine pathway.

Reactive metabolites from Glyceraldehyde-3-phosphate

Increased production of superoxide by respiratory chain due to increased blood glucose activates formation of advanced glycation end products, causes inhibition of glyceraldehyde-3-phosphate dehydrogenase and disrupts extracellular function.³⁰ Though glyceraldehyde is not a major sugar, its concentration is increased during diabetic state, and oxidative stress due to inhibition of GAPDH that leads to the formation of GLAP. GLAP-RAGE elicits formation

of free radicals, inflammation and thrombogenic reaction in HUVECs.

Prevention of binding of GLAP –RAGE would be a novel mechanism of treatment of vascular injury in diabetes. Inhibition of GAPDH has been reported to cause accumulation of dicarbonyls like methylglyoxal (MG), derived from triosephosphates.³¹ GAPDH activity in the kidneys of the diabetic rats was less than the normal controls.³² Antisense DNA mediated inhibition of GAPDH, activated major pathways of hyperglycemic damage similar to elevated blood glucose levels. GAPDH activity is modified by ADP-ribose polymerization due to increased superoxide and is inhibited.

Reactive species from oxidized - LDL

Uptake of oxidized LDL by scavenger receptor in macrophages causes formation of foam cells and atherosclerosis.³³ Fluidity of cell membranes is modified due to lipid peroxidation and disturbs cellular function.³⁴ Elevated levels of oxidized LDL increase the expression of the transmembrane LOX-1 receptor which induces high NADPH oxidase activity that leads to oxidative pathways.³⁵ Pyruvate in addition to free fatty acids also causes local production of reactive oxygen species. Lipid peroxides present within LDL accelerates heme mediated oxidation of LDL. Enhanced expression of mitochondrial superoxide dismutase (mSOD) is activated on binding of oxidized low-density lipoproteins (Ox-LDL) to receptors on monocytes, macrophages and smooth muscle cells. Increased action of superoxide dismutase generates higher levels of H₂O₂. Lipoxygenase mediated oxidation of LDL enhances in the presence of H₂O₂ which is inhibited by the presence of lipoxygenase inhibitors. Lipoxygenase activity is triggered in the presence of specific stimuli or general tissue injury.³⁶ In type 2 diabetes, increased oxidative stress occurs in cells and tissues and glycation of low density lipoprotein makes it prone to events that enhances subsequent oxidative modification. Several lines of evidence support a proatherogenic role for oxidized LDL (Ox-LDL) and its in vivo existence.³⁷

Conclusion

Intervention of the diabetic complications require identification of a antioxidant target site at the juncture of the link between various reactive metabolites (Oxygen, carbon, nitrogen) using omic techniques at the genome, transcriptome or proteome level to prevent its activation, and switching off hyperglycemic memory to decrease production of reactive species in mitochondria.

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Conflict of interest

The author declares no conflict of interest.

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