Erythrocyte oxidative stress in clinical management of diabetes and its cardiovascular complications

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Accepted: 13 September 2006

ABSTRACT

Diabetes mellitus is a chronic disease in its own right and is also regarded as a cardiovascular risk factor as well as a cardiovascular disease, due to its ability to progress to a stage of cardiovascular co-morbidity. The pathophysiology of cardiovascular complications in diabetes is reported to involve hyperglycaemia-induced oxidative stress. The erythrocyte has an array of endogenous antioxidants involved in quenching oxidant production and the exponential chain reactions in diabetes. When the erythrocyte is oxidatively stressed, as demonstrated by depleted reduced glutathione and/or increased malondialdehyde in its cell membrane, the risk of diabetes progression and its cardiovascular sequelae, including atherosclerosis and coronary artery disease, is increased. Virtually all studies that determined erythrocyte malondialdehyde and glutathione in diabetes show consistently increased and reduced levels, respectively. Furthermore, cardiovascular complications of diabetes are reported to commence at the prediabetes stage. Current coronary artery disease screening programmes based on the presence of two or more risk factors are failing to identify those with increased risk of diabetes and cardiovascular complications, thereby limiting early interventions. Screening that includes erythrocyte oxidative stress determination may provide an additional marker for both preclinical and advanced disease. In this review, a concise description of the involvement of erythrocyte oxidative stress in diabetes mellitus and its cardiovascular sequelae is presented. Antioxidant action and interaction in the erythrocyte are also described, with emphasis on why current coronary artery disease screening markers cannot be regarded as erythrocyte oxidative stress markers.

KEY WORDS: Cardiovascular diseases.
Diabetes complications.
Diabetes mellitus.
Erythrocytes.
Oxidative stress.

Introduction

Oxidative stress (OS) is a disturbance in the oxidant/antioxidant balance, in favour of oxidants. It is a state in which a cell experiences alteration of cellular components, due to exposure to free radicals and reactive oxygen species (ROS) beyond its antioxidant capacity.[1] Although ROS are essential for cellular functions, such as the utilisation of nutrient chemical energy in the production of adenosine triphosphate (ATP), excess oxidant exposes the cells involved to OS.[2]

Erythrocyte oxidative stress

Erythrocyte oxidant stress (EOS) is a type of cellular oxidative stress involving functional impairment of red blood cells,[3] which arises from over-exposure of the cellular components to various ROS. Erythrocyte functional mechanisms are overwhelmed by alterations in the normal metabolic and/or physiological activities that generate ROS, due to the oxidant challenge exceeding the red cell antioxidant-producing capacity.[4,5] Antioxidant capacity is indicated by several biomarkers (e.g., reduced glutathione [GSH] and methaemoglobin [metHb]).

There are three possible sources of ROS. First, erythrocytes can become oxidatively stressed by normal physiological processes.[2] Owing to the role of erythrocytes in oxygen transport and the presence of redox-active haemoglobin molecules, they generate relatively high levels of ROS, with their attendant deleterious effect of oxidative stress.[6] Furthermore, incomplete reduction of oxygen in the mitochondrial electron transport system resulting in $O_2^{\cdot-}$ generation also occurs.[7] Although there are no mitochondria in erythrocytes, there is the risk that $O_2^{\cdot-}$ will enter the erythrocyte membrane through superoxide channels,[8] especially during hyperglycaemia and/or dyslipidaemia.[5]

Second, there is hyperglycaemia-induced generation of ROS,[8] which causes oxidative damage to the erythrocyte.[9]

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The erythrocyte functions by utilising glucose to generate ATP via glycolysis and the pentose phosphate pathway (PPP). The increased rate of glycolysis, to meet the cellular need for ATP, as in diabetes mellitus (DM), is associated with increased free radical generation, which depletes GSH content.[10] The PPP produces reduced nicotinamide adenine dinucleotide phosphate (NADPH), which is a potent electron donor during GSH regeneration, but over-production of NADPH could drive the production of $O_2^{\cdot-}$ radicals, thereby exacerbating oxidative stress.[5,11]

Third, in metHb reduction to haemoglobin, the GSH level is depleted as it converts to oxidised glutathione (GSSG),[12] leading to reduced erythrocyte antioxidant capacity and, consequently, oxidative stress. Methaemoglobin reductase is also involved in the reduction of Fe^{3+}, but this pathway uses up NADPH, which is a requirement for GSH reductase (GR) activity for the regeneration of GSH from GSSG.[13] Thus, either pathway of metHb reduction results in decreased erythrocyte GSH level, which translates to antioxidant imbalance in the cell.

Erythrocyte oxidant stress and cardiovascular disease

Accumulating research reports demonstrate changes in erythrocyte antioxidant and haem components in diabetes complications such as cardiovascular disease (CVD).[9,14-19] The generation of ROS in erythrocytes, coupled with depletion of its defensive natural antioxidants, enhances activation of the nuclear redox-sensitive transcription factor.[8] This results in up-regulation of events at the gene level, such as pro-coagulant tissue factors and pro-inflammatory mediators that lead to endothelial dysfunction and CVD.[20] Hence, EOS may affect certain cardiovascular events (Fig. 1), including hypercoagulation[21] and endothelial dysfunction.[19,22]

Erythrocyte oxidant stress decreases erythrocyte membrane deformability,[23] which is one of the factors that influence blood flow/shear rate, which can lead to thrombotic events.[24] Nevertheless, while there is consensus that atherosclerosis represents increased oxidative stress, which contributes to the clinical manifestations of coronary artery disease (CAD), it has yet to be shown that oxidative modification causes atherosclerosis and CAD.[25]

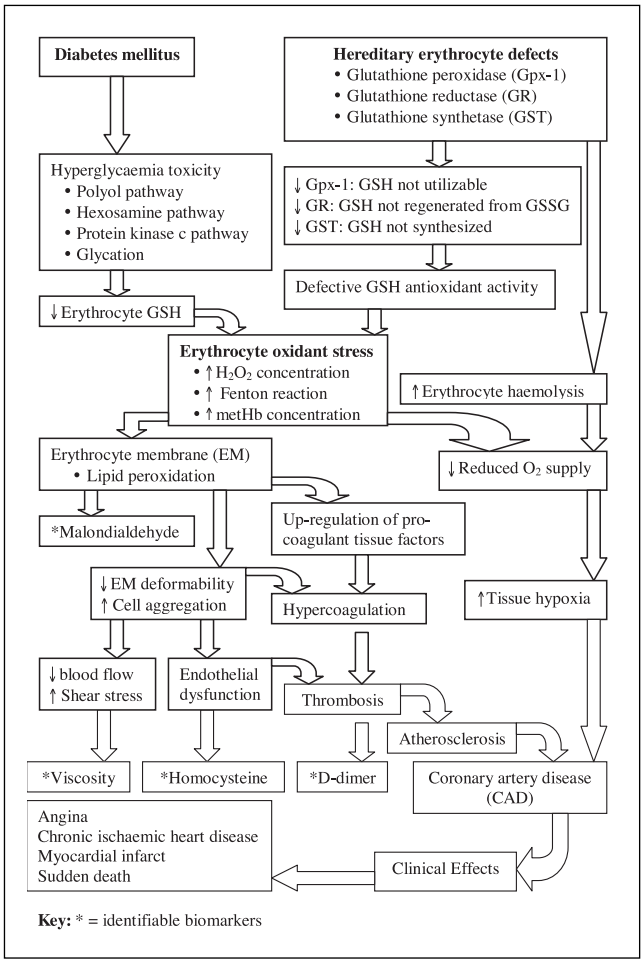

Fig. 1. Illustration of links between EOS and related cardiovascular biomarkers/events.

Blood viscosity

Blood viscosity is the intrinsic resistance of blood to flow in the vascular system. During normal blood flow, erythrocyte deformability (physiological changing of shape) enables the cell to adapt physically and squeeze past an otherwise rough vascular wall or viscous space.[26,27] When EOS occurs through lipid peroxidation of low-density lipoprotein (LDL) content, the cell membrane becomes rigid, less deformable and less adaptable.[28] This makes the blood more viscous and results in vascular abnormalities such as increased red cell aggregation, which are seen in atherosclerosis and CAD.[23]

Associated blood flow abnormalities can be detected primarily at low shear rate as increased viscosity. However, there is a school of thought that believes the increase in blood viscosity in individuals affected by ischaemia is due to decreasing erythrocyte deformability.[29] Thus, whether decreased deformability leads to ischaemia or vice versa has yet to be addressed. Moreover, some studies of viscosity used plasma,[30,31] while some used whole blood.[21,32] Therefore, the clinical utility of blood viscosity and the choice of specimen-associated EOS has yet to be determined.

Endothelial dysfunction

Of emerging interest is hyperglycaemia-induced endothelial dysfunction.[33,34] The oxidation of LDL, as seen in diabetic dyslipidaemia, initiates a chronic inflammatory reaction that results in endothelial damage, culminating in atherosclerosis and CAD.[35]

There is speculation that oxidant/antioxidant imbalance results in endothelial dysfunction,[36] which is marked by increased plasma homocysteine level and other biomarkers.[22] However, homocysteine is known to competitively inhibit GSH synthesis at the cysteine-dependent synthesis pathway,[9,22,37] and/or enhance generation of $O_2^{\cdot-}$ and H_2O_2 radicals,[38] which results in OS. Therefore, the way that endothelial damage and OS interact remains unclear.

Coagulation and fibrinolysis

Several studies report an association between D-dimer, which indicates fibrinolysis, and CVD and DM.[39] For instance, Sommeijer *et al*.[40] report that some coagulation markers are elevated in DM. A preliminary report[41] from the authors' laboratory also shows increased D-dimer levels in DM. However, Yano *et al*.[42] suggest that there is hypo-fibrinolysis as thrombomodulin-thrombin complex in type 2 diabetes. This is formed on intact vascular endothelium, and may activate thrombin-activatable fibrinolysis inhibitor (TAFI). This suggestion is supported by the observation that hyperglycaemia and insulin enhance the synthesis and secretion of PAI-1.[43]

These proposals suggest that fibrinolysis, and therefore the generation of D-dimer, is reduced in DM. Nevertheless, the generation of EOS leads to up-regulation of pro-

coagulant tissue factors at the gene level,[8] suggesting that D-dimer changes in diabetes have not been explained adequately.

Haemolytic anaemia

Deficiencies of GSH peroxidase (GPx-1), GR and GSH synthetase enzymes, which, respectively, utilise, regenerate and synthesise GSH, have long been known as rare hereditary erythrocyte defects that cause non-spherocytic haemolytic anaemia.[44] Thus, the involvement of the erythrocyte in cardiovascular complications of DM can also be viewed in association with haemolytic anaemia and/or reduced oxygen supply associated with changes in red blood cell morphology.[29,45] Hyperglycaemia, which results in reduced GSH via the polyol pathway,[8] coupled with the inability of GR/GST to regenerate/synthesise GSH,[13] increases haemolysis.[4] The effect is a sequence of anaemia, reduced blood/nutrient/O_2 supply, ischaemia and subsequent angina, chronic ischaemic heart disease, myocardial infarct or sudden death.[45]

Oxidative stress as a target in the management of diabetes and its cardiovascular complications

It is known that development or progression of diabetes can be delayed, and that the key to successful prevention and/or treatment is early diagnosis and targeted treatment.[46]

Diagnosis

The diagnosis of DM is based on symptoms, including polyuria, polydipsia and unexplained weight loss, plus random blood glucose level ≥11.0 mmol/L; fasting blood glucose level ≥7.0 mmol/L; or oral glucose tolerance test (OGTT) ≥11.0 mmol/L at two-hour post-glucose load of 75 g at fasting.[47]

Diagnosis of cardiovascular complications of DM is difficult, especially in the asymptomatic or early stages.[48] Laboratory tests include the traditional cholesterol profile and glucose,[49-51] while emerging markers for CAD in diabetes include C-reactive protein (CRP), D-dimer and homocysteine.[52,53]

C-reactive protein is a marker of low-grade inflammation that may reflect the inflammatory aspect of atherosclerosis, particularly when CRP levels increase dramatically during acute inflammatory episodes. However, in view of the very low incidence of high CRP levels in the absence of borderline or abnormal levels of established risk factors, the extent of CRP involvement in CVD remains controversial.[54]

Although moderately elevated plasma homocysteine level is associated with increased risk of CVD,[55] association of homocysteine with CVD complications in DM requires further study, especially in type 2 DM.[56] In type 1 DM, higher plasma homocysteine levels have been reported,[57,58] while lower and similar levels compared with controls have also been reported.[22]

Several studies have been carried out on erythrocyte GSH and malondialdehyde (MDA) status in diabetes as oxidative stress indices and reports have been consistent. For instance, Memisogullari *et al*.[18] studied the levels of serum antioxidant proteins and erythrocyte antioxidant and oxidant biomarkers in patients with type 2 diabetes, with and without complications. They found, for example, that levels of erythrocyte MDA were significantly increased and positively associated with glucose levels in diabetes groups, whereas erythrocyte GSH was significantly decreased.

Dominguez *et al*. determined the presence of oxidative stress in early type I diabetes,[16] and showed that MDA is increased while erythrocyte GSH levels are decreased at onset of the disease. In another study,[59] lipid peroxidation in the erythrocyte cell membrane was determined in prediabetes using erythrocyte GSH and MDA as indices. The results indicate a significant reduction in GSH and elevation of MDA. Preliminary results from the authors' laboratory demonstrate that erythrocyte MDA is higher[60] and erythrocyte GSH is lower in diabetes and prediabetes.[61]

In summary, there is evidence of observable changes in EOS indices in DM and its cardiovascular sequelae. However, controversy remains about which biomarker is most reliable for clinical diagnostic use. It may be more appropriate to use a panel of tests that include antioxidant and OS markers.[62]

Management

Most management measures are devoted to modification of risk factors such as diet, obesity, physical inactivity[63] and smoking.[64] Among medication options available, preservation of normal oxidant-antioxidant balance is considered important for preventing CVD progression.[65] Hence, anti-inflammatory and hypoglycaemic medication, as well as statins, are used in the management of diabetes and CVD, but there is growing interest in their antioxidant activities,[66,67] as are drugs such as vitamins A, C and E, as well as coenzyme-Q supplements, which are used primarily as antioxidants.[68-70]

Antioxidants

Antioxidants prevent oxidation of cell components by donating an electron to the free radicals that initiate or take part in oxidative reactions.[71] Oxidant stability is achieved by removal of electrons from surrounding molecules to produce an electron pair. However, the molecule that loses the electron then possesses an unpaired electron and becomes another free radical. If the reactivity is high, further target molecules are attacked. Thus, a single radical can initiate a sequence of electron transfer (redox) reactions. When the antioxidant becomes the target, the resultant radical will possess a low reactivity and the chain reaction is broken.[7]

Depending on their source, there are three types of antioxidants. The endogenous group of antioxidants is found naturally in cells. In erythrocytes this group includes catalase, GSH, GPx-1, metHb reductase, NADPH and SOD. Catalase, GPx-1 and SOD are central to erythrocyte antioxidant function.[71]

The nutritional group of antioxidants, obtained from the diet as micronutrients, include vitamin C (ascorbic acid) and vitamin E (tocopherol).[36] Others are micronutrients such as β-carotene, cysteine, flavonoids (e.g., quercetin), polyphenols, as well as the trace metals zinc, selenium, manganese and copper[7] Sources of such molecules include citrus fruits, strawberries, papaya, red pepper and broccoli for vitamin C; vegetable oils, whole grain cereals and eggs

Table 1. Classes of antioxidant based on mode of action.

Antioxidant enzymes	Chain breakers		Metal-binding proteins
	Aqueous phase chain breakers	Lipid phase chain breakers	
Catalase	GSH/GSSG	Carotenoids	Albumin
GPx-1	Vitamin C	Flavonoids	Ceruloplasmin
GR		Ubiquinol-10	Ferritin
SOD		Vitamin E	Lactoferrin
			Transferrin

GPx-1: Glutathione peroxidase; GR: Glutathione reductase; SOD: superoxide dismutase.

for vitamin E; carrots, potatoes, pumpkin, spinach, apricots, broccoli and green vegetables for carotenoids; and apples and tea for flavonoids.[72]

Finally, there are the antioxidant supplements. This group includes pharmaceutical products such as assorted brands of over-the-counter coenzyme Q, selenium, vitamins and additives to foods and creams.[73]

Mode of antioxidant action

Maxwell and Lip[7] and Young and Woodside[74] classified antioxidants into three groups based on their mode of action (Table 1). Superoxide dismutase first catalyses the conversion of O_2^- to H_2O_2 (Equation 1). The H_2O_2 is then reduced to water by either catalase or GPx-1 (Equations 2 and 3).[7,74] When these enzymes fail to neutralise O_2^- and H_2O_2 adequately, the Fenton reaction and membrane lipid-peroxidation occur, resulting in the formation of OH^- and lipid peroxyl radicals.[12,75]

Specific to erythrocytes, the Fenton reaction results in methaemoglobinaemia.[12,17,76] The limitation to GPx-1 activity is the propensity of GSH regeneration (Equation 4) by GR to maintain a normal GSH/GSSH ratio. This reaction is an important antioxidant mechanism in preventing oxidative stress that arises from the depletion of GSH (Equation 3).[8]

Chain-breaking antioxidants

A chain reaction[74] occurs when an oxidant reacts with a molecule and a new radical is formed. The new radical then reacts with another molecule to produce yet another radical, and this continues exponentially until the radical reacts with a molecule (i.e., chain-breaking antioxidant) that forms a stable product.[1]

Besides acting as substrate in enzyme-catalysed antioxidation, GSH can follow three other pathways along which it acts as a chain-breaking antioxidant. One pathway is the reduction of metHb to haemoglobin (Fig. 2 ¶[3]). This is important in the prevention of the Fenton reaction (Fig. 2 ¶[7]).[12] The second pathway involves the lipid peroxidation process. Following erythrocyte membrane lipid peroxidation, vitamin E (α-tocopherol) performs antioxidation by donating one electron (via an H radical) to the resultant lipid peroxyl radical to form lipid peroxide and tocopheroxyl radicals (Equation 5). The lipid peroxide then reacts with GSH to produce a stable lipid-OH (Equation 6).[74] The third antioxidant activity of GSH is indirect regeneration of other antioxidants. Tocopheroxyl (tocopherol-$O^\cdot$) that was formed in Equation 5 can be reduced by GSH (Equation 7).

Both vitamin C (Equation 8) and coenzyme Q (Equation 9) regenerate tocopherol radicals.[74] The regeneration of tocopherol via the vitamin E regeneration system (VERS) by coenzyme-Q, reduced glutathione and/or vitamin C from its oxidant form (tocopheroxyl radical) is quite complex. Nevertheless, in the absence of any of the VERS components, tocopheroxyl exhibits a pro-oxidant property in a process called tocopherol-mediated peroxidation.[70] Thus, in the absence of adequate dietary micronutrients (vitamin C and CoQ-H_2), GSH gains importance in preventing vitamin E from exhibiting its pro-oxidant effect.

The antioxidation function of vitamin C is the regeneration of vitamin E (Equation 8).[77] It occurs at the

EQUATIONS

$2O_2^- + 2H^+ \xrightarrow{\text{Superoxide dismutase}} H_2O_2 + O_2$ EQ 1

$2H_2O_2 \xrightarrow{\text{Catalase}} 2H_2O + O_2$ EQ 2

$H_2O_2 + 2GSH \xrightarrow{\text{Gpx-1}} GSSG + 2H_2O$ EQ 3

$GSSG + NADPH + H^+ \xrightarrow{\text{Glutathione reductase}} 2GSH + NADP^+$ EQ 4

$\text{Lipid-}O_2^\cdot + \text{Tocopherol-OH} \rightarrow \text{Lipid-}O_2H + \text{Tocopherol-}O^\cdot$ EQ 5

$\text{Lipid-}O_2H + 2GSH \rightarrow \text{Lipid-OH} + GSSG + H_2O$ EQ 6

$2\text{Tocopherol-}O^\cdot + 2GSH \rightarrow 2\text{Tocopherol-OH} + GSSG$ EQ 7

$2\text{Tocopherol-}O^\cdot + \text{Ascorbic acid} \rightarrow \text{Dehydro-ascorbic acid} + 2\text{Tocopherol-OH}$ EQ 8

$2\text{Tocopherol-}O^\cdot + \text{CoQ-}H_2 \rightarrow 2\text{Tocopherol-OH} + \text{CoQ}$ EQ 9

$\text{Dehydro-ascorbic acid} + 2GSH \rightarrow \text{Ascorbic acid} + GSSG$ EQ 10

$2\text{Lipid-}O_2^\cdot + \text{CoQ-}H_2 \rightarrow 2\text{Lipid-}O_2H + \text{CoQ}$ EQ 11

aqueous phase and involves two single-electron reductions, initially to yield semi-dehydro-ascorbyl radicals and later dehydroascorbate. The dehydroascorbate is reconverted to ascorbic acid by GSH in the reaction (Equation 10).[25, 78]

Ubiquinol (CoQ-H_2) follows both a direct and an indirect antioxidation pathway. Indirectly, it acts by regenerating α-tocopherol (Equation 9). Directly, it could break the lipid peroxidation chain by the donation of electrons, and become ubiquinone (Equation 11).[78]

The ubiquinone (CoQ) formed in Equations 9 and 11 is reconverted to ubiquinol in the respiratory chain. Paradoxically, this ubiquinol/ubiquinone metabolism in the respiratory phase is associated with superoxide production, making ubiquinol both an antioxidant and a pro-oxidant.[78]

Transition metal-binding protein antioxidants

The transition metal-binding proteins act by preventing copper and iron from binding with hydroxyl radicals. Albumin and ceruloplasmin bind to copper, while ferritin, lactoferrin and transferrin bind to iron, thereby stabilising them.[7] One important property suggested is that the presence of some antioxidants might lead to increased oxidative stress, particularly if copper and iron are present.[17]

Antioxidant interactions and supplement

Overall, complex interactions exist between antioxidants (Fig. 2).[7,74] The nature of oxidant injury or the free radical responsible for OS will determine whether or not the prevailing antioxidant breaks the exponential chain reaction.[74,79,80] In diabetes, the initial oxidant injury on erythrocytes is via $O_2^{\cdot-}$, followed by its product of dismutation, H_2O_2. The prevailing antioxidant is GSH and its associated enzymes. Figure 2 illustrates some of the main antioxidant interactions that occur in the erythrocyte.

There are at least five reaction processes (Fig. 2, ¶[1] - ¶[5]) that influence GSH concentration in the erythrocyte. These involve four metabolites, including homocysteine and dismuted ROS,[38] which directly affect erythrocyte GSH status. Virtually all antioxidants and oxidants are involved in competitive, simultaneous interactions. Therefore, the importance of a particular antioxidant depends on the micro and macro environment at a specific time, and on the nature of the oxidant-induced injury.[74]

For example, a review[80] of changes in oxidative stress biomarkers in diabetes, their consequences and the effect of conventional and alternative drugs shows that effective antioxidant activity depends on the type of oxidant and diabetic complication. Another study[79] examined whether or not high glucose levels lead to disruption in glutathione-dependent antioxidant defences, and capacity to handle oxidative stress was tested. The results indicate that glucose/hyperglycaemia toxicity reduces erythrocyte GSH and, at a certain low GSH level, the maximum GR activity to regenerate GSH is insufficient to restore normality. The implication is that GSH is a factor in diabetes complications.[15] Thus, low erythrocyte GSH level in diabetes requires appropriate supplementation with a GSH precursor such as cysteine to improve erythrocyte GSH synthesis and maintenance.[38]

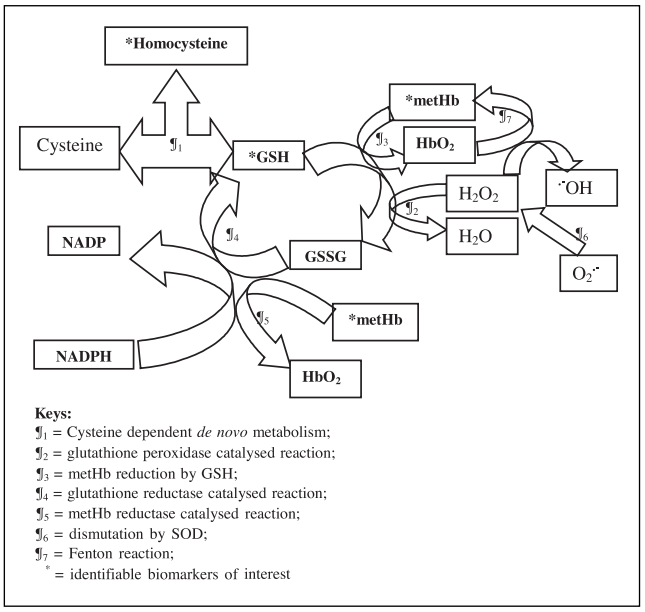

Fig. 2. Schematic illustration of glutathione interactions with other antioxidants in the erythrocyte.[20,41]

Complications of diabetes and cardiovascular disease: an overview of an oxidative stress link

Diabetes mellitus, which includes insulin-dependent (type I), non-insulin-dependent (type II) and gestational diabetes, is a common chronic disease associated with devastating complications that include CVD.[81]

Hyperglycaemia is proposed as the basis for the increased occurrence of CVD complications in DM.[82,83] Thus, as the occurrence of DM increases, so too do its cardiovascular complications, which are the leading cause of morbidity and mortality in persons with DM.[5,81] Hence, DM is associated with the early phase of CVD, and diabetes patients need to be screened regularly for early identification of CVD.[51]

There are individuals who do not have established DM, yet show higher than normal blood glucose levels.[84] Such individuals have prediabetes, characterised either by an impaired fasting blood glucose level of 5.6–6.9 mmol/L,[85] or impaired glucose tolerance blood glucose level of 7.0–10.9 mmol/L.[86] It is now well established that persons with prediabetes are at great risk of developing CVD,[87-90] in addition to developing diabetes. Hence, improving early detection of prediabetes could reduce the human and economic costs of DM.[91,92]

The criteria of using two or more risk factors is not helping to identify asymptomatic patients with a higher prevalence of CAD.[93] Therefore, an aggressive diagnostic approach in those with preclinical DM associated with risk factors for CAD is imperative, and monitoring of oxidative stress status in prediabetes may become an important adjunct strategy.[61]

Cardiovascular complication of diabetes

The mechanisms associated with CVD development among persons with diabetes and prediabetes vary and include hyperglycaemia, family history and dyslipidaemia.[94] The identification of risk factors associated with preclinical

hyperglycaemia is important in reducing mortality and morbidity associated with diabetes complications.[95]

Hyperglycaemia can mediate its adverse effects through pathways that include polyol, hexosamine, protein kinase C and glycation,[34] the unifying feature of these pathways being the over-production of the oxidant superoxide ion ($O2^{\cdot-}$), which increases susceptibility to intracellular oxidative stress.[5,8] Oxidative stress is enhanced by diabetic dyslipidaemia characterised by increased oxidation of membrane lipids,[96] which exacerbates atherosclerosis.[5,97] Hence, it is recommended that persons with established diabetes be screened annually and treated for hyperlipidaemia if LDL-cholesterol level exceeds 3.38 mmol/L.[51]

Metabolic syndrome is another factor. It is related to insulin resistance and is characterised by albuminuria, dyslipidaemia, inflammation, hypercoagulability, hypertension and obesity.[98] It is regarded as a distinct disease, but is now appraised as a state of co-existing cardiovascular risk factors.[99] Nevertheless, metabolic syndrome includes pathophysiological factors associated with oxidative stress, and requires early identification in order to reduce the disease burden.

The development of atherosclerosis and CAD involves blood clot formation. Glucose and insulin have the capacity to enhance the synthesis and secretion of plasminogen-activator inhibitor type 1 (PAI-1),[43] which promotes the stability and extension of clot formation. Thus, diabetes enhances the development of deep vein thrombosis. Furthermore, insulin resistance causes the release of free fatty acid (FFA) from adipocytes into arterial endothelial cells, leading to increased FFA oxidation and the over-production of ROS including $O_2^{\cdot-}$, hydrogen peroxide (H_2O_2) and hydroxyl ($OH^{\cdot}$) radicals.[8] Thus, there is evidence that oxidative stress is a unifying mechanism in the development of cardiovascular complications associated with diabetes.[100]

Conclusions

Current healthcare and preventative medicine does not identify a large proportion of people with prediabetes, yet they are at significant risk of CVD, which requires early intervention. This review emphasises the fact that oxidative stress and/or antioxidant deficiencies are involved in the onset of diabetes and its cardiovascular sequelae. As erythrocyte levels of MDA and GSH are determinants of oxidative stress and antioxidant status in diabetes, antioxidants can be an effective treatment against oxidative stress.

Biomarkers of oxidative stress status and related cardiovascular events are not used in routine clinical practice, as antioxidant interactions in the erythrocyte have not been given proper consideration in the interpretation and use of oxidative stress evaluations in research. Thus, tests are required to determine whether oxidant activities are high and/or what antioxidant is deficient and requires supplementation.[90]

It is therefore important to determine (i) how the levels of antioxidant and oxidative stress biomarkers, plus related cardiovascular events, differ between normal and pathological conditions, and in stages in the progression of diabetes (including prediabetes); and (ii) how the identifiable changes in biomarkers relate as a panel of tests in non-diabetes and diabetes.

The findings could prove useful for the screening and management of OS in prediabetes, which will help in early identification and/or intervention, improved patient care and reduced morbidity and mortality. □

This review was made possible by research approval and sponsorship from Charles Sturt University (CSU). Ezekiel Nwose was in receipt of a CSU postgraduate scholarship. Herbert Jelinek and Ross Richards were in receipt of a CSU Community of Scholars grant.

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