Review: Targeting therapeutics against glutathione depletion in diabetes and its complications

Callum Livingstone and James Davis

British Journal of Diabetes & Vascular Disease 2007 7: 258
DOI: 10.1177/14746514070070060201

Targeting therapeutics against glutathione depletion in diabetes and its complications

CALLUM LIVINGSTONE,[1] JAMES DAVIS[2]

Abstract

Glutathione (GSH) is the most abundant intracellular antioxidant, the dysregulation of which is widely implicated in disease states. There is *in vitro* and clinical evidence that abnormal glutathione status is involved in β-cell dysfunction and in the pathogenesis of long-term complications of diabetes. Interest has developed in the potential for therapeutic modification of glutathione status in the treatment of diabetes. There is evidence which supports the use of glutathione pro-drugs, lipoic acid and vitamin supplementation but further studies are required before these enter widespread use. Studies into the role of oxidative stress in diabetes rely heavily on the ability to measure glutathione, which has been a problematic analyte to measure in the laboratory. New electrochemical methods being developed should speed up the rate at which data can be accumulated and will help define clinical utility for its measurement.

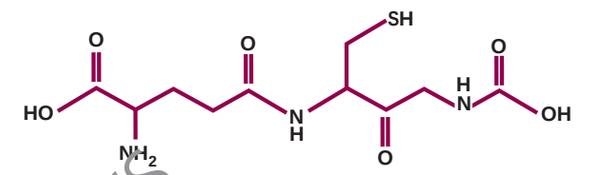
Br J Diabetes Vasc Dis 2007;**7**:258–65

Key words: complications, diabetes, glutathione, lipoic acid.

Introduction

The tripeptide glutathione (GSH, glutamylcysteinylglycine) (figure 1) is the most abundant intracellular non-protein thiol, occupying an important place in cellular metabolism as an antioxidant. It is found ubiquitously in animal cells present at millimolar concentrations predominantly in the reduced state. Some 20% of intracellular glutathione is located in the mitochondria where it helps protect against ROS produced as by-products of the electron transport chain. Glutathione is abundant in dietary sources with fruits and vegetables contributing over 50% of intake, though most of the body's requirements are synthesised *de novo* from its constituent amino acids. It is predominantly synthesised in hepatocytes from which it is exported to peripheral tissues.[1] Glutathione itself is not readily taken up by the cells of these tissues. Instead, plasma membrane bound GGT must first cleave it into its constituent amino acids which are then transported across the plasma membrane. The rate limiting step in glutathione biosynthesis is catalysed by the enzyme GCL which can be modulated by Nrf-2 regulated expression.[1] Cysteine is the limiting substrate.[3]

Figure 1. Glutathione (L-γ-glutamyl-L-cysteinyl-glycine)



Glutathione has a variety of crucial physiological roles outlined in figure 2. First and foremost, it is the central member of a complex antioxidant system protecting the cell from oxidative stress.[4] It is a cofactor for GPx which is a defence mechanism against peroxides, preventing the accumulation of ROS and so preventing cellular injury. GSSG is formed as a product of the detoxification. GSH is regenerated by the action of GR, an NADPH-dependent enzyme, thus completing the redox cycle. Normally about 99% of intracellular glutathione exists in the reduced form. The GSH system is exquisitely sensitive to changes in this ratio and maintains it very efficiently, indeed almost thermostatically. GSH is also a cofactor for the enzyme dehydroascorbate reductase, which recycles dehydroascorbate back to reduced ascorbic acid (vitamin C). Glutathione therefore maintains vitamins C and E in their reduced forms by the reversible oxidation of its sulphydryl group.[5] Secondly, GSH serves as a cofactor in conjugation reactions mediated by GSTs which protect macromolecules against toxic xenobiotics such as drugs and carcinogens. The adducts formed are then actively secreted from the cell. Whilst this is beneficial in ridding the cell of toxins, it depletes the cellular glutathione pool. Thirdly, glutathione modulates the redox status of thiol groups in signalling proteins, through which it can influence a variety of cell functions e.g. transcription, gene expression,[6] cell proliferation[7,8] and programmed cell death[9] though the precise mechanisms are unknown. Glutathione itself is known to translocate into the nucleus and may participate directly in the regulation of gene transcription.

[1] Clinical Biochemistry Department, Royal Surrey County Hospital, Guildford, Surrey, GU2 7XX, UK.
[2] Department of Chemistry, School of Biomedical and Natural Sciences, Nottingham Trent University, Nottingham, NG11 8NS, UK.

Correspondence to: Dr Callum Livingstone
Consultant Chemical Pathologist, Clinical Biochemistry Department, Royal Surrey County Hospital, Guildford, Surrey, GU2 7XX, UK.
Tel: +44 (0)1483 464121; Fax: +44 (0)1483 464072
E-mail: clivingstone@royalsurrey.nhs.uk

Abbreviations

CHD	coronary heart disease
DHAR	dehydroascorbate reductase
DNA	deoxyribonucleic acid
H_2O_2	hydrogen peroxide
HbSSG	glutathionylated haemoglobin
HPLC	high performance liquid chromatography
GCL	glutamate-L-cysteine ligase
GCS	γ-glutamylcysteine synthetase
GGT	γ-glutamyltransferase
GLUTs	glucose transporters
GPx	glutathione peroxidase
GR	glutathione reductase
GRX	glutaredoxin
GSH	reduced glutathione
GST	glutathione-S-transferase
GSSG	oxidised glutathione or glutathione disulphide
mRNA	messenger ribonucleic acid
NAC	N-acetylcysteine
NADP	nicotinamide adenine dinucleotide phosphate
NADPH	nicotinamide adenine dinucleotide phosphate, reduced form
NF-κB	nuclear factor –κb
Nrf-2	nuclear factor E2-related factor-2
OTC	2-oxothiazolidine-4-carboxylate
O_2^-	superoxide anion
RAGE	receptor for advanced glycation end products
ROO·	peroxyl radical
ROOH	membrane lipid
ROS	reactive oxygen species
SOD	superoxide dismutase
SSG	gluthione adduct to protein

Glutathione and oxidative stress

The term 'oxidative stress' describes the situation where there is an imbalance between ROS and the defence mechanisms designed to remove them. In oxidative stress, abnormalities in the redox state may lead to disease by disturbance of the cellular processes listed above. For example, the transcription factor NF-κB regulates a number of genes involved in immune and inflammatory responses.[10] It is activated by agents which generate a pro-oxidant state.[11] The cytotoxic effects of ROS are likely to contribute to the pathogenesis of many chronic diseases. This goes hand-in-hand with low levels of glutathione. Considerable evidence has built up from clinical studies to support a role for glutathione depletion in disease. Population studies have indicated that health and longevity are related to glutathione levels in blood.[12] In a study on patients with a variety of chronic diseases over a wide age range, significant glutathione depletion was noted in 36% of patients, with the decrease being accounted for by low concentrations of GSH while the GSSG concentration remained unchanged.[13]

Diabetes

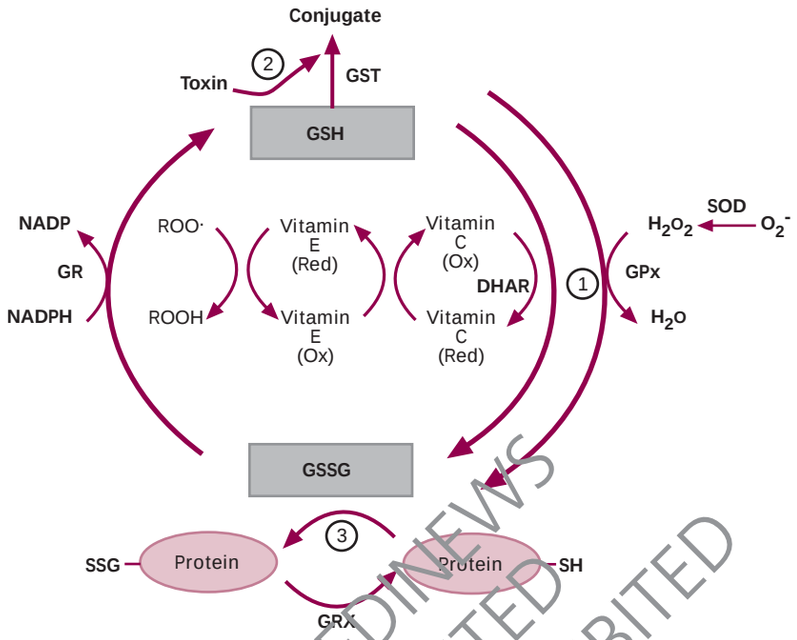
It is well established that there is a link between oxidative stress and diabetes and recent research has aimed to clarify the role of glutathione, a number of studies having investigated its level in blood from patients. Erythrocyte glutathione levels were reported to be low in patients with type 2 diabetes, possibly due to impaired activity of the enzyme GCS which is involved in the biosynthesis of glutathione.[9,14] Studies on these patients have also reported increased levels of GSSG due to both decreased GR activity and decreased transport of GSSG out of the cells.[15,16] It would appear that patients with diabetes tend to have a smaller and more oxidised glutathione pool than control subjects of similar age. This impaired glutathione metabolism would be expected to weaken the defence against oxidative stress. Similar changes in glutathione status have been observed in ageing in the absence of disease, which may be in part explained by age-related decrease in plasma vitamin C and E.[16-21] Ageing patients with diabetes will have these changes superimposed on diabetes-related changes and so it is anticipated that they would be more susceptible to oxidative stress.

There are several mechanisms whereby hyperglycaemia may bring about oxidative stress via changes in glutathione metabolism (figure 3).[22] Firstly, excessive glucose oxidation overloading the mitochondrial electron transport chain is thought to be the main source of O_2^- putting demands upon the glutathione pool. Hyperglycaemia also results in increased flux through the polyol pathway causing NADPH depletion, impaired GR activity and a decrease in the GSH:GSSG ratio. There is increased formation of advanced glycation end-products which are known to bind to RAGE receptors thereby generating ROS and depleting glutathione.[23,24] *In vitro* studies on cultured cells have demonstrated that exposure to high extracellular glucose concentrations leads to a decrease in intracellular glutathione levels proposed to result from reduced activity of GCS and enzymatic glycation.[9,25,26] There is also decreased GPx activity, which is expected to increase oxidative stress. Hyperglycaemia does not require to be sustained in order to promote oxidative stress. Even brief hyperglycaemic episodes which occur during a glucose tolerance test or postprandially can decrease the antioxidant capacity of plasma in normal and diabetic subjects.[27,28]

Beta cells

It is well recognised that type 2 diabetes is a progressive condition with insulin production tending to fall with time as the β-cell mass falls and the cells synthesise less insulin. Hyperglycaemia is recognised to have toxic effects on the β-cell, so-called 'glucotoxicity' leading to reduced insulin gene expression, impaired insulin secretion and ultimately cell death.[29] Recently it has been hypothesised that chronic oxidative stress as a consequence of hyperglycaemia is an important mechanism for glucotoxicity.[30] The mechanisms whereby glucose can lead to the production of ROS in β-cells are well defined. β-cells appear to be vulnerable to oxidative stress as they contain relatively low levels of GPx and other protective enzymes compared to other cells.[31] Moreover, studies on pancreatic islets and β-cell lines observed the cells to be incapable of increasing their antioxidant enzyme expression in response to cellular stress induced by exposure to glucose.[32] There is evidence that ROS are also involved in the pathogenesis of peripheral insulin resistance.[33]

Figure 2. Roles of glutathione in cell physiology



Key: The roles of glutathione shown are 1. antioxidant, 2. antitoxic and 3. modulator. GSH = glutathione (reduced form); GSSG = glutathione (oxidised form); GR = glutathione reductase; GPx = glutathione peroxidase; GST = glutathione-S-transferase; SOD = superoxide dismutase; DHAR = dehydroascorbate reductase; GRX = glutaredoxin; Ox = oxidised form; Red = reduced form; NADP = nicotinamide adenine dinucleotide phosphate; NADPH = nicotinamide adenine dinucleotide phosphate, reduced form; SSG = gluthione adduct to protein; ROO· = peroxyl radical; ROOH = membrane lipid; SH = thiol group; H_2O_2 = hydrogen peroxide; O_2- = superoxide anion

Nitrosative stress is also thought to contribute to the pathogenesis of β-cell apoptosis.[34] Over time these factors will increase the burden on β-cells, promoting the development of diabetes.

Macrovascular complications

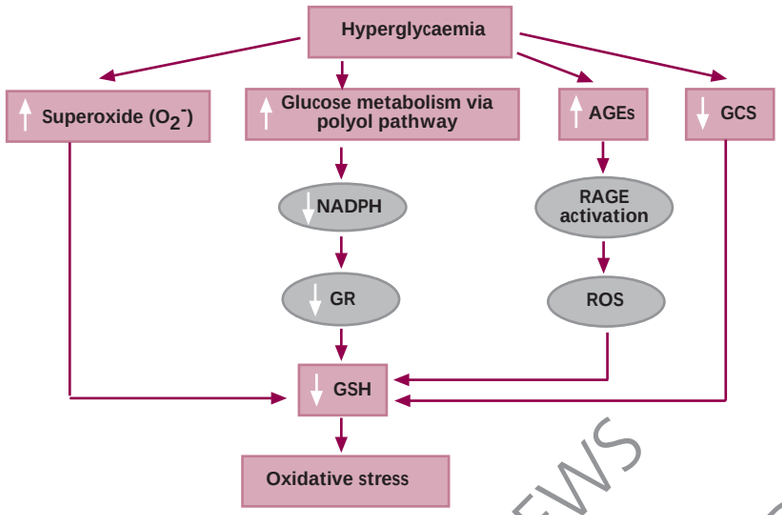
There is considerable interest in the role of oxidative stress in development of the long-term complications of diabetes. Measurements of oxidative stress correlate closely with their development.[35,36] ROS appear to cause endothelial cell dysfunction which precedes atherosclerosis and so contributes to the initiation of CHD, the main cause of mortality in patients with type 2 diabetes.[37-39] Considering the central role occupied by glutathione in antioxidant defence, its depletion is causally implicated in this process. Weakened antioxidant defence with regard to diminished GPx and GR activities has been reported to be present in human atherosclerotic disease.[40] In a recent study comparing patients with advanced CHD with age-matched controls, the patients showed reductions in red blood cell GPx and SOD activities which were more marked than the degree of hyperlipidaemia.[41] Further work will clarify whether there is utility for the measurement of these parameters in the clinical assessment of cardiovascular risk.

Microvasular complications

Evidence is accumulating that oxidative stress contributes to the development of microvascular complications of diabetes.[42] Glutathione is implicated in the pathogenesis of all these complications. Under normal circumstances, the eye contains high concentrations of GSH, GR and GPx but changes in these occur throughout the eye in diabetes. Decreased activity of GPx and lower levels of ascorbic acid were noted in the eye lens of patients with diabetes, especially in those with diabetic retinopathy.[43] Plasma GSH concentrations were significantly lower in these patients than in control subjects. *In vitro* studies have investigated the causes of these changes. Chronic exposure of retinal cells to high concentrations of glucose caused decreased synthesis of glutathione, reducing the size of the GSH pool[44] and the enzymes of glutathione metabolism were inactivated by glycation as was GSH itself. The role of GSH and related enzymes in the eye has recently been reviewed.[45]

Many patients with diabetes and even impaired glucose tolerance develop neuropathic complications despite good overall glycaemic control. Short periods of postprandial hyperglycaemia appear to generate sufficient oxidative stress to be damaging to neurones. Glucose enters neurones by a concentration dependent transport mechanism so these cells are particularly susceptible to adverse effects of hyperglycaemia and oxidative stress. There is considerable *in vitro* evidence for a central role of glutathione in diabetic neuropathy.[46] Experimental glutathione depletion of neuronal cells renders them susceptible to oxidative injury and cell death.[47] Conversely, glutathione loading of neuronal cells can prevent apoptosis in response to cytotoxic stimuli.[48] Glutathione parameters also

Figure 3. Mechanisms whereby hyperglycaemia leads to oxidative stress



Key: GSH = glutathione (reduced form); GR = glutathione reductase; AGEs = advanced glycation end-products; GCS = γ-glutamylcysteine synthetase; NADP = nicotinamide adenine dinucleotide phosphate; NADPH =nicotinamide adenine dinucleotide phosphate, reduced form; O_2- = superoxide anion; RAGE = receptor for advanced glycation end products; ROS = reactive oxygen species

appear to be deranged in patients with diabetic nephropathy. A recent study on diabetic patients with microalbuminuria noted the study group to have significantly lower red blood cell GSH and GPx levels than diabetic subjects without microalbuminuria.[49]

Non-enzymatic glycation of proteins is implicated in the pathophysiology of microvascular complications. Patients with microvascular disease are reported to have a higher proportion of HbSSG compared to patients without complications or to control subjects.[50] The HbSSG level correlated with the duration of diabetes, measures of glycaemic control and oxidative stress. It was negatively correlated with the red blood cell GSH level suggesting that enhanced oxidative stress in diabetes may result in increased protein glutathionylation, having an adverse effect on cellular glutathione levels. Taken together, these findings suggest a likely role for glutathione deficiency in the development of microvascular complications but further clinical studies are clearly needed to clarify this role.

Therapeutic implications

It is clear from the above that there is a strong theoretical basis for believing that the therapeutic elevation of intracellular glutathione levels would be beneficial in patients with diabetes. In fact a considerable body of evidence already supports the therapeutic potential of antioxidant thiols.[51] Most of the reports of beneficial effects are in model systems with as yet relatively little data from clinical studies. Various approaches have been taken to enhancing glutathione levels. Its oral administration has been observed to increase plasma glutathione levels. However, as a therapeutic agent glutathione suffers from the limitation that it is not readily taken up by cells and so direct supplementation is an inefficient means of delivery.[15] Consequently, alternative approaches have been developed for exogenous supplementation of intracellular glutathione levels. In contrast to their parent compound, glutathione esters are readily transported into cells where they are hydrolysed to yield free glutathione and have potential for the treatment of its deficiency.[52] Repletion of intracellular glutathione has been achieved with both monoesters and diesters i.e. esterified at the glycine residue.[53] There is evidence for a role in preventing nephrotoxicity and cataract.[54,55]

Cysteine prodrugs

Cysteine, the limiting substrate for glutathione synthesis, can be supplied as the precursor NAC which is deacetylated after transport into cells. It has long been used as an antidote for paracetamol overdose by preventing liver damage resulting from glutathione depletion. NAC can inhibit the action of the transcription factor NF-κB discussed above which is believed to be involved in the mechanism whereby oxidative stress leads to cellular pathology.[56] There is considerable evidence from *in vitro* and animal model studies on diabetic rats and mice for the beneficial effects of NAC in diabetes. Treatment of cultured lens cells with NAC decreased hyperglycaemia-induced oxidation of lens proteins.[57] NAC treatment of streptozotocin-induced diabetic animals reduced glutathione oxidation,[58] lipid peroxidation,[59] inflammatory cytokine levels[60] and increased fibrinolytic factor concentrations.[60] There is also evidence from studies of an animal model of type 2 diabetes that NAC treatment can ameliorate the development of insulin resistance amongst other beneficial effects.[33]

If the oxidative stress hypothesis of glucotoxicity is correct, it would be anticipated that early intervention with antioxidant therapy would prevent the decline in β-cell mass and integrity, so modifying the sequence of events leading to diabetes. There

is evidence that this is the case. Over-expression of GPx and GCL, an enzyme involved in the biosynthesis of glutathione, protects islet cells against the adverse effects of pro-oxidants.[61,62] In addition, NAC treatment of islet β-cells partly prevents the defects in cell function associated with oxidative stress *viz* reduced insulin mRNA, DNA binding of transcription factors, cellular insulin content and glucose-induced insulin secretion.[63] Subsequent application of this treatment to animal models of type 2 diabetes prevented hyperglycaemia and defective β-cell function by maintaining expression of the insulin gene as well as β-cell insulin content and secretion. More recently, treatment with NAC has been observed to improve β-cell function in an animal model of type 1 diabetes.[64,65]

Another cysteine pro-drug, OTC, is readily transported into cells where it can increase glutathione levels.[66] Oral supplementation of OTC has been observed to enhance endothelial integrity in patients with coronary artery disease and so has a potential therapeutic role in diabetes.[67] In considering the therapeutic benefit of thiol antioxidants, a potential drawback should be borne in mind. It is likely that excessive thiol levels would have a detrimental effect on redox sensitive signalling pathways and so it would be advisable not to exceed the plasma free thiol concentrations expected in a young healthy adult i.e. about 16 μM.

Vitamins

Vitamins E and C work in tandem with glutathione to remove ROS. They therefore need to be included in any discussion of the therapeutic role of glutathione as their supplementation along with other nutrients potentially has a role in limiting ROS-mediated damage. Dietary deficiency of vitamin E causes reduced GPx and GR activity, both of which are restored by vitamin E supplementation.[68] Vitamin E supplementation also increases the plasma level of GSH as would be expected from its place in metabolism.[69] Vitamin C deficiency results in decreased plasma GSH levels as a result of decreased conversion of GSSG back to the reduced form.[70] Deficits of both vitamins C and E have been reported in diabetes.[42] The consideration of vitamin C status is complicated by the observation that lymphocyte vitamin C levels may be low in patients with diabetes, in the face of normal plasma levels.[71] This intracellular vitamin C deficiency may be explained by competition with glucose for cellular uptake or down regulation of GLUTs. *In vitro* work has demonstrated that glucose competitively inhibits leucocyte uptake of dehydroascorbic acid, even at glucose levels in the physiological range.[72]

Whilst correction of deficiencies is a valid therapeutic approach, it is less clear whether supplementation above normal intake reduces the risk of disease. This will be important to clarify as there is concern that excessive supplementation of some antioxidants may be hazardous e.g. selenium and vitamin C combined with iron.[73,74] Oral therapy with vitamins C and E significantly improved endothelial function in type 1 diabetes[75] and there is evidence that a high dietary intake of vitamin E is associated with decreased low density lipoprotein oxidisability and reduced risk of mortality from CHD.[76,77] In patients with type 2 diabetes oral supplementation with vitamins C and E and folic acid was observed to benefit antioxidant parameters including plasma GSH and GPx levels and lipid oxidation.[78-80] Although there is evidence that vitamin supplementation can improve oxidative stress parameters in diabetes this is a complex area with much conflicting data.[75,81-2] Recently a meta-analysis examined 68 randomised controlled trials of the use of antioxidant supplements for the prevention of disease.[83] Overall no significant effect on mortality was observed. When 47 low-bias trials were considered separately β-carotene, vitamin A and vitamin E supplementation were associated with an increase in mortality. In a recent review, the authors concluded that there were insufficient data to recommend ingestion of antioxidant vitamins in excess of recommended dietary allowance.[84]

Trace metals

GPx is a selenoprotein and copper, zinc and manganese are all required for forms of SOD. Magnesium is a cofactor for GR. Dietary deficiencies of these metals results in reduced clearance of ROS, with peroxidative damage to proteins.[85] Metal deficiencies have been reported to be commoner in patients with type 2 diabetes as compared to age-matched controls.[86] Patients with disorders predisposing to trace element deficiencies should have their trace metal status checked periodically and supplemented as necessary. More studies are required on trace metal status in diabetes.

Exercise

High intensity training up-regulates muscle SOD and GPx enhancing the ability of the individual to deal with the increased ROS generation that accompanies exercise.[87] In studies on streptozotocin-induced diabetic animals, exercise combined with supplementation of vitamins A and E improved levels of antioxidant enzymes and glutathione in the kidney and eyes.[88] Exercised animals also appear to have a greater overall antioxidant status and suffer smaller myocardial infarctions following ischaemia-reperfusion injury.[89] A recent human study compared the oxidative stress profile between sedentary and physically active subjects with type 2 diabetes. Although total glutathione levels were similar between the groups, the physically active subjects had more favourable oxidative stress and haemodynamic profiles.[90] Further work is needed to investigate exercise-induced changes in glutathione parameters in patients with diabetes.

Lipoic acid

Lipoic acid is a sulphur-containing compound synthesised *de novo* in humans though it is usually present in adequate amounts in the diet.[91] Its main physiological function is as a co-enzyme for the pyruvate dehydrogenase multienzyme complex in mitochondria. Lipoic acid has some ROS-scavenging properties but works mainly through its reduced form dihydrolipoic acid which is a more potent antioxidant than GSH. As well as scavenging ROS, Lipoic acid is involved in recycling endogenous antioxidants, such as vitamins C and E, and helps prevent oxidative processes by chelating metal cations. It interacts

Key messages

- Glutathione is the most abundant intracellular thiol antioxidant
- Glutathione has a central role in defence against reactive oxygen species
- Oxidative stress is involved in the development and progression of diabetes and its complications. Glutathione is strongly implicated in these processes
- Preliminary evidence suggests modifying glutathione status may benefit patients with diabetes

directly with the GSH system as a substrate for GR and so facilitates regeneration of GSH.[92]

Administration of lipoic acid causes a rise in the cellular level of GSH, GPx activity and vitamins C and E.[93] When given to diabetic patients with poor glycaemic control, it can improve antioxidant defence, reduce oxidative stress and improve the microcirculation and it has been shown to decrease protein glycation rate.[94,95] Considerable interest has therefore developed in its potential use in preventing microvascular complications. Clinical trial evidence for the benefit of lipoic acid is accumulating. A meta-analysis of studies on its effect in patients with diabetic neuropathy concluded that short-term treatment (three weeks) with lipoic acid 600 mg o.d. administered intravenously significantly reduced symptoms of diabetic polyneuropathy and improved underlying deficits.[96] Longer-term oral treatment (four to seven months) reduced neuropathic deficits and improved cardiac autonomic neuropathy. A long-term multicentre trial of oral treatment (Neurological Assessment of Thioctic Acid in Neuropathy study [NATHAN I]) is currently underway in the USA. Lipoic acid is licensed in Germany for use in patients with diabetes but is not yet licensed for use in the USA or UK.

Measurement of glutathione

The most established method for measuring thiols is Ellman's method described in 1959.[97] It is based on the thiol-disulphide exchange reaction between dithionitrobenzoic acid and thiols. Thiols react with this agent to give a yellow colour measured spectophotometrically at 412 nm. Whilst GSH can be detected using this method, it doesn't distinguish between individual thiol-containing molecules. Methods later evolved for the specific determination of GSH. The levels of both GSH and GSSG can be measured using a GR cycling method which follows the oxidation of NADPH to NADP by measuring absorbance at 340 nm.[98] A number of HPLC methods have been described. Iodoacetic acid can be used to form derivatives of thiols with subsequent conversion of the free amino groups to 2,4 dinitrophenyl derivatives and HPLC separation.[99] More recently a post-column derivatisation method was described, capable of selectively distinguishing between monomolecular thiols.[100] There is also an assay method which uses derivatisation and reverse phase ion-pair liquid chromatography.[101] It can measure reduced, oxidised and protein-bound forms of the monomolecular thiols present in human plasma, including glutathione.

Whilst HPLC methods are highly sensitive and specific for GSH they do not overcome some inherent problems with its measurement. Free plasma glutathione levels are very low (micromolar range) compared to total thiol levels. About 99.5% of blood glutathione is contained in red blood cells with the consequence that even a tiny degree of haemolysis or leakage will result in a large artefact. GSH is also rapidly oxidised to GSSG and equally rapidly forms mixed disulphides, so its half-life in plasma is less than two minutes. The plasma glutathione level is also subject to some diurnal variation with levels being rather lower during fasting.[19] Inevitably, as a result of these problems, there have been wide differences in levels reported between studies. Researchers measuring GSH should be aware of these pitfalls and keep interferences to a minimum in particular by carrying out rapid analysis of specimens and seeking to avoid haemolysis.

The difficulty and expense in measuring glutathione has undoubtedly been a limiting factor in research. If more is to be learned about the role of glutathione in diabetes novel methods will be required for its measurement. The authors have developed an electrochemical method for measuring total thiols on plasma in patients with diabetes and are currently developing methodology for the specific measurement of glutathione on whole blood.[102,103] The beauty of electrochemistry in this and other contexts is its rapidity, small specimen requirement and potential for miniaturisation into a near patient-testing device. Such methodology will allow rapid generation of data on glutathione levels enabling its clinical utility to be established.

Future directions

Antioxidants do not work in isolation. Future studies will therefore need to consider the effect of combination therapies with different antioxidant treatments and with existing anti-diabetic therapies. The potential impact of antioxidant therapies on diabetes and its complications is exciting. Studies into such therapies will be greatly facilitated by the ability to measure glutathione and it is likely that monitoring of glutathione levels will prove advisable during therapy and risk assessment.

References

1. Deneke SM, Fanburg BL. Regulation of cellular glutathione. *Am J Physiol Lung Cell Mol Physiol* 1989;**257**:E790-E800.
2. Okouchi M, Okayama N, Alexander JS, Aw TY. NRF2-dependent glutamate-L-cysteine ligase catalytic subunit expression mediates insulin protection against hyperglycaemia-induced brain endothelial cell apoptosis. *Curr Neurovasc Res* 2006;**3**:249-61.
3. Ji LL. Exercise and oxidative stress: role of the cellular antioxidant systems. *Exercise Sport Sci Rev* 1995;**23**:135-66.
4. Pompella A, Visvikis A, Paolicchi A, DeTata V, Casini AF. The changing faces of glutathione, a cellular protagonist. *Biochem Pharmacol* 2003;**66**:1499-503.
5. Reed DJ. Glutathione: toxicologic implications. *Ann Rev Pharmacol Toxicol* 1990;**30**:603-31.

6. Arrigo AP. Gene expression and thiol redox state. *Free Rad Biol Med* 1999;**27**:936-44.
7. Hwang C, Sinskey AJ. The role of oxidation-reduction potential in monitoring growth of mammalian cultured cells. In: Spier RE, Griffiths JB, Meignier B eds Production of biologicals from animal cells in culture. Oxford: Halley Court;1991:548-67.
8. Gardiner CS, Reed DJ. Synthesis of glutathione in the preimplantation mouse embryo. *Arch Biochem Biophys* 1995;**318**:30-6.
9. Yoshida K, Hirokawa J, Tagami S, Kawakami Y, Urata Y, Kondo T. Weakened cellular scavenging activity against oxidative stress in diabetes mellitus: regulation of glutathione synthesis and efflux. *Diabetologia* 1995;**38**:201-10.
10. Baeuerle P, Baltimore D. NF-kappa B: ten years after. *Cell* 1996;**87**:13-20.
11. Schreck R, Albermann K, Baeuerle PA. Nuclear factor kappa B: an oxidative stress-responsive transcription factor of eukaryotic cells. *Free Rad Res Commun* 1991;**17**:221-37.
12. Julius M, Lang CA, Gleiberman L, Harburg E, DiFrancisco W, Schork MA. Glutathione and morbidity in a community based sample of elderly. *J Clin Epidemiol* 1994;**47**:1021-6.
13. Lang CA, Mills BJ, Mastropaolo W, Liu MC. Blood glutathione decreases in chronic diseases. *J Lab Clin Med* 2000;**135**:402-05.
14. Murakami K, Kondo T, Ohtsuka Y, Fujiwara Y, Shimada M, Kawakami Y. Impairment of glutathione metabolism in erythrocytes from patients with diabetes mellitus. *Metabolism* 1989;**38**:753-8.
15. Forrester TE, Badaloo V, Bennett FI, Jackson AA. Excessive excretion of 5-oxoproloine and decreased levels of blood glutathione in type II diabetes mellitus. *Eur J Clin Nutr* 1990;**44**:847-50.
16. Samiec P, Drews-Botsch C, Flagg EF *et al*. Glutathione in human plasma: decline in association with ageing, age-related macular degeneration and diabetes. *Free Rad Biol Med* 1998;**24**:699-704.
17. Lang CA, Naryshkin S, Schneider DL, Mills BJ, Lindeman RD. Low blood glutathione levels in healthy ageing adults. *J Lab Clin Med* 1992; **120**:720-5.
18. Flagg EW, Coates RJ, Jones DP *et al.* Plasma total glutathione in humans and its association with demographic and health-related factors. *Br J Nutr* 1993;**70**:797-808.
19. Erden-Inal M, Sunal E, Kanbak G. Age related changes in the glutathione redox system. *Cell Biochem Funct* 2002;**20**:61-6.
20. Rikans LE, Moore DR. Effect of ageing on aqueous phase antioxidants in tissues of male Fischer rats. *Biochem Biophys Acta* 1988;**966**:269-75.
21. Vandewoude MFJ, Vandewoude MG. Vitamin E status in normal population: the influence of age. *J Am Coll Nutr* 1987;**6**:207-11.
22. Bayraktutan U. Free radicals, diabetes and endothelial dysfunction. *Diab Obes Metab* 2002;**4**:224-38.
23. Deuther-Conrad W, Loske C, Schinzel R, Dringen R, Riederer P, Munch G. Advanced glycation end products change glutathione redox status in SH-SY5Y human neuroblastoma cells by a hydrogen peroxide dependent mechanism. *Neurosci Lett* 2001;**312**:29-32.
24. Loske C, Neumann A, Cunningham AM *et al.* Cytotoxicity of advanced glycation end-products is mediated by oxidative stress. *J Neural Transm* 1998;**105**:1005-15.
25. Sharpe PC, Liu WH, Yue KKM *et al.* Glucose induced oxidative stress in vascular contractile cells. *Diabetes* 1998;**47**:801-09.
26. Lu SC. Regulation of hepatic glutathione synthesis. Current concepts and controversies. *FASEB J* 1999;**13**:1169-83.
27. Ceriello A, Bortolotti N, Crescentini A *et al.* Antioxidant defences are reduced during the oral glucose tolerance test in normal and non-insulin-dependent diabetic subjects. *Eur J Clin Invest* 1998;**28**:329-33.
28. Ceriello A, Bortolotti N, Motz E *et al.* Meal induced oxidative stress and low density lipoprotein oxidation in diabetes: the possible role of hyperglycaemia. *Metabolism* 1999;**48**:1503-08.
29. Robertson RP. Chronic oxidative stress as a central mechanism for glucose toxicity in pancreatic islet beta cells in diabetes. *J Biol Chem* 2004;**279**:42351-4.
30. Robertson RP, Harmon J, Tran PO, Tanaka Y, Takahashi H. Glucose toxicity in beta cells: type-2 diabetes, good radicals gone bad and the glutathione connection. *Diabetes* 2003;**52**:581-7.
31. Grankvist K, Marklund SL, Taljedal IB. CuZn-superoxide dismutase, Mn-superoxide dismutase, catalase and glutathione peroxidase in pancreatic islets and other tissues in the mouse. *Biochem J* 1981;**199**:393-8.
32. Tiedge M, Lortz S, Drinkgern A, Lenzen S. Relation between antioxidant enzyme gene expression and antioxidative defense status of insulin-producing cells. *Diabetes* 1997;**46**:1733-42.
33. Haber CA, Lam TK, Yu Z *et al.* N-acetylcysteine and taurine prevent hyperglycaemia-induced insulin resistance *in vivo*: possible role of oxidative stress. *Am J Physiol Endocrinol Metab* 2003;**285**:744-53.
34. Bast A, Wolf G, Oberbaumer I, Walther R. Oxidative and nitrosative stress induces peroxiredoxins in pancreatic beta cells. *Diabeteologia* 2002;**45**:867-76.
35. Zaltzberg H, Kanter Y, Aviram M, Levy Y. Increased plasma oxidizability and decreased erythrocyte and plasma antioxidative capacity in patients with NIDDM. *Isr Med Assoc J* 1999;**1**:228-31.
36. Sundaram RK, Bhaskar A, Vijayalingam S, Viswanathan M, Mohan R, Shanmugasundaram KR. Antioxidant status and lipid peroxidation in type 2 diabetes mellitus with and without complications. *Clin Sci* 1996;**90**:255-60.
37. Tesfamariam B. Free radicals in diabetic endothelial dysfunction. *Free Radic Biol Med* 1994;**16**:383-91.
38. Lyons TJ. Oxidised low density lipoproteins: a role in the pathogenesis of atherosclerosis in diabetes. *Diabet Med* 1991;**8**:411-19.
39. Elhadd TA, Kennedy G, Hill A *et al.* Abnormal markers of endothelial cell activation and oxidative stress in children, adolescenys and young adults with type 1 diabetes with no clinical vascular disease. *Diab Metab Rese Rev* 1999;**15**:405-11.
40. Lapenna D, DeGioia S, Ciofani G *et al.* Glutathione related antioxidant defences in human atherosclerotic plaques. *Circulation* 1998; **97**:1930-4.
41. Zawadzka-Bartczak E. Activities of red blood cell anti-oxidative enzymes (SOD and GPx) and total anti-oxidative capacity of serum (TAS) in men with coronary atherosclerosis and healthy pilots. *Med Sci Monit* 2005;**11**:440-4.
42. Vincent AM, Russell JW, Low P, Feldman EL. Oxidative stress in the pathogenesis of diabetic neuropathy. *Endocr Rev* 2004;**25**:612-18.
43. Altomare E, Grattagliano I, Vendemaile G, Micelli-Ferrari T, Signorile A, Cardia L. Oxidative protein damage in human diabetic eye: evidence of a retinal participation. *Eur J Clin Invest* 1997;**27**:141-7.
44. Lu SC, Bao Y, Huang ZZ, Sarthy VP, Kannan R. Regulation of gamma-glutamylcysteine synthetase subunit gene expression in retinal Muller cells by oxidative stress. *Invest Ophthalmol Vis Sci* 1999;**40**:1776-82.
45. Ganea E, Harding JJ. Glutathione-related enzymes and the eye. *Curr Eye Res* 2006;**31**:1-11.
46. Pop-Busui R, Sima A, Stevens M. Diabetic neuropathy and oxidative stress. *Diab Metab Res Rev* 2006;**22**:257-73.
47. Rizzardini M, Lupi M, Bernasconi S, Mangolini A, Cantoni L. Mitochondrial dysfunction and death in motor nerones exposed to the glutathione-depleting agent ethacrynic acid. *J Neurol Sci* 2003;**207**:51-8.
48. Kobayashi MS, Han D, Packer L. Antioxidants and herbal extracts protect HT-4 neuronal cells against glutamate-induced cytotoxicity. *Free Radic Res* 2000;**32**:115-24.
49. Ozdemir G, Ozden M, Maral H, Kuskay S, Cetinalp P, Tarkun I. Malonaldehyde, glutathione, glutathione peroxidase and homocysteine levels in type 2 diabetic patients with and without microalbuminuria. *Ann Clin Biochem* 2005;**42**:99-104.
50. Sampathkumar R, Balasubramanyam M, Suarsal S, Rema M, Mohan V, Balaram P. Increased glutathionylated hemoglobin (HbSSG) in type 2 diabetes subjects with microangiopathy. *Clin Biochem* 2005;**38**:892-9.
51. Sen CK. Redox signalling and the emerging therapeutic potential of thiol antioxidants. *Biochem Pharmacol* 1998;**55**:1747-58.
52. Anderson ME, Levy EJ, Meister A. Preparation and use of glutathione monoesters. *Meth Enzymol* 1994;**234**:492.
53. Levy EJ, Anderson ME, Meister A. Transport of glutathione diethyl ester into human cells. *Proc Natl Acad Sci USA* 1993;**90**:9171-5.
54. Babu E, Ebrahim AS, Chandramohan N, Sakthisekaran D. Rehabilitating role of glutathione ester on cisplatin induce nephrotoxicity. *Ren Fail* 1999;**21**:209-17.
55. Blunt S, Takemoto L. Inhibition of selenite cataract by S-diethylsuccinyl glutathione isopropyl ester. *Curr Eye Res* 2000;**20**:341-5.
56. Mihm S, Galter D, Droge W. Modulation of transcription factor NF-kB activity by intracellular glutathione levels and by variations of the extracellular cysteine supply. *FASEB J* 1995;**9**:246-52.

57. Jain AK, Lim G, Langford M, Jain SK. Effect of high-glucose levels on protein oxidation in cultured lens cells and in crystalline and albumin solution and its inhibition by vitamin B6 and N-acetylcysteine: its possible relevance to cataract formation in diabetes. *Free Radic Biol Med* 2002;**33**:1615-21.
58. Pariarca S, Furfaro AL, Domenicotti C *et al.* Supplementation with N-acetylcysteine and taurine failed to restore glutathione content in liver of streptozotocin-induced diabetic rats but protected against oxidative stress. *Biochem Biophys Acta* 2005;**1741**:48-54.
59. Xia Z, Nagareddy PR, Guo Z, Zhang W, McNeill JH. Antioxidant N-acetylcysteine restores systematic nitric oxide availability and corrects depressions in arterial blood pressure and heart rate in diabetic rats. *Free Radic Res* 2006;**40**:175-84.
60. Hsu CC, Yen HF, Yin MC, Tsai CM, Hsieh CH. Five cysteine-containing compounds delay diabetic deterioration in Balb/cA mice. *J Nutr* 2004;**134**:3245-9.
61. Tanaka Y, Tran PO, Harmon J, Robertson RP. A role for glutathione peroxidase in protecting pancreatic beta cells against oxidative stress in a model of glucose toxicity. *Proc Natl Acad Sci USA* 2002;**99**:12363-8.
62. Sakai K, Matsumoto K, Nishikawa T *et al.* Mitochondrial reactive oxygen species reduce insulin secretion by pancreatic beta cells. *Biochem Biophys Res Commun* 2003;**300**:216-22.
63. Tanaka Y, Gleason CE, Tran PO, Harmon JS, Robertson RP. Prevention of glucose toxicity in HIT-T15 cells and Zucker diabetic fatty rats by antioxidants. *Proc Natl Acad Sci USA* 1999;**96**:10857-62.
64. Takatori A, Ishii Y, Itagaki S, Kyuwa S, Yoshikawa Y. Amelioration of the beta cell dysfunction in diabetic APA hamsters by antioxidants and AGE inhibitor treatments. *Diab Metab Res Rev* 2004;**20**:211-18.
65. Kaneto H, Kajimoto Y, Miyagawa J *et al.* Beneficial effects of antioxidants in diabetes: possible protection of pancreatic beta cells against glucose toxicity. *Diabetes* 1999;**48**:2398-406.
66. Porta P, Aebi S, Summer K, Lauterberg BH. L-2-Oxothiazolidine-4-carboxylic acid, a cysteine prodrug: pharmacokinetics and effects on thiols in plasma and lymphocytes in humans. *Pharmacol Exp Ther* 1991;**257**:331-4.
67. De Vita JA, Frei B, Holbrook M *et al.* L-2-oxothiazolidine-4-carboxylic acid reverses endothelial dysfunction in patients with coronary artery disease. *J Clin Invest* 1998;**101**:1408-114.
68. Chow CK, Reddy K, Tappel A. Effect of dietary vitamin E on the activities of glutathione peroxidase in rat tissues. *J Nutr* 1973;**103**:618-24.
69. Costagliola C, Menzione M. Effect of vitamin E on the oxidative state of glutathione in plasma. *Clin Physiol Biochem* 1990;**8**:140-3.
70. Henning SM, Zhang JZ, McKee RW, Swenseid ME, Jacob RA. Glutathione blood levels and other oxidant defence indices in men fed with diets low in vitamin C. *J Nutr* 1991;**121**:1969-75.
71. Yamada H, Waki M, Yamada Y, Umegaki K. Lymphocyte and plasma vitamin C levels in type-2 diabetic patients with and without diabetic complications. *Diab Care* 2004;**27**:2491-2.
72. Ngkeekwong FC, Ng LL. Two distict uptake mechanisms for ascorbate and dehydroascorbate in human lymphoblasts and their interaction with glucose. *Biochem J* 1997;**324**:225-30.
73. Goldhaber SB. Trace element risk assessment: essentiality versus toxicity. *Regul Toxicol Pharmacol* 2003;**38**:232-42.
74. Fisher AEO, Naughton DP. Iron supplements: the quick fix with long term consequences. *Nutr J* 2004;**3**:2-6.
75. Beckman JA, Goldfine AB, Gordon MB, Garrett LA, Keaney JF, Creager MA. Oral antioxidant therapy improves endothelial function in type-1 but not type-2 diabetes mellitus. *Am J Physiol Heart Circ Physiol* 2003;**285**:H2392-H2398.
76. Marangon K, Devaraj S, Tirosh O, Packer L, Jialal I. Comparison of the effect of alpha-lipoic acid and alpha tocopherol supplementation on measures of oxidative stress. *Free Rad Biol Med* 1999;**27**:1114-21.
77. Rimm EB, Stampfer MJ, Ascherio A, Giovannucci E, Colditz GA, Willett WC. Vitamin E consumption and the risk of coronary heart disease in men. *NEJM* 1993;**328**:1450-6.
78. Naziroglu M, Simsek M, Simsek H, Aydilek N, Ozcan Z, Atilgan R. The effects of hormone replacement therapy combined with vitamins C and E on antioxidant levels and lipid profiles in post- menopausal women with type 2 diabetes. *Clin Chem Acta* 2004;**344**:63-71.
79. Ble-Castillo JL, Carmona-Diaz E, Mendez JD *et al.* Effect of alpha-tocopherol on the metabolic control and oxidative stress in female type 2 diabetics. *Biomed Pharmacother* 2005;**59**:290-5.
80. Child DF, Hudson PR, Jones H *et al.* The effect of oral folic acid on glutathione, glycaemia and lipids in type 2 diabetes. *Diab Nutr Metab* 2004;**17**:95-102.
81. Fuller CJ, Chandalia M, Garg A, Grundy SM, Jialal I. RRR-alpha tocopherol acetate supplementation at pharmacologic doses decreases low density lipoprotein oxidative susceptibility but not protein glycation in patients with diabetes mellitus. *Am J Clin Nutr* 1996; **63**:753-9.
82. Li D, Devaraj S, Fuller C, Bucala R, Jialal I. Effect of alpha-tocopherol on LDL oxidation and glycation: *in vitro* and *in vivo* studies. *J Lipid Res* 1996;**37**:1978-86.
83. Bjelakovic G, Nikolova D, Gluud LL, Simonetti RG, Gluud C. Mortality in randomised trials of antioxidant supplements for primary and secondary prevention: systematic review and meta-analysis. *JAMA* 2007;**297**:842-57.
84. Hasanain B, Mooradian AD. Antioxidant vitamins and their influence in diabetes mellitus. *Curr Diab Rep* 2002;**2**:448-56.
85. Aruoma OI. Free radicals, oxidative stress and antioxidants in human health and disease. *J Am Chem Soc* 1998;**75**:199.
86. Havivi E, Baron H, Reshef A, Stein P, Raz I. Vitamins and trace metals status in non insulin dependent diabetes. *Int J Vitam Nutr Res* 1991;**61**:328-33.
87. Powers SK, Lennon SL. Analysis of cellular responses to free radicals: focus on exercise and skeletal muscle. *Proc Nut Soc* 1999;**58**:1025-33.
88. Kutlu M, Naziroglu M, Simsek H, Yilmaz T, Sahap Kukner A. Moderate exercise combined with dietary vitamins C and E counteracts oxidative stress in the kidney and lens of streptozotocin-induced diabetic rat. *Int J Vitam Nutr Res* 2005;**75**:71-80.
89. Hamilton KL, Staib JL, Phillips T, Hess A, Lennon SL, Powers SK. Exercise, antioxidants, and HSP72: protection against myocardial ischaemia/reperfusion. *Free Radic Biol Med* 2003;**34**:800-09.
90. Villa-Caballero L, Nava-Ocampo AA, Frati-Munari AC *et al*. Hemodynamic and oxidative stress profile after exercise in type 2 diabetes. *Diab Res Clin Pract* 2006 (Epub ahead of print).
91. Biska A, Wlodek L. Lipoic acid – the drug of the future? *Pharm Rep* 2005;**57**:570-7.
92. Atmaca G. Antioxidant effects of sulphur containing amino acids. *Yons Med J* 2004;**45**:776-88.
93. Maitra I, Serbinova E, Trischler H, Packer L. Alpha lipoic acid prevents buthionine sulfoxime-induced cataract formation in newborn rats. *Free Radic Biol Med* 1995;**18**:823-9.
94. Borcea V, Nourooz-Zadeh J, Wolff SP *et al.* Alpha lipoic acid decreases oxidative stress in diabetic patients with poor glycaemic control and albuminuria. *Free Radic Biol Med* 2000;**26**:1495-500.
95. Schleicher E, Wagner E, Nehrlich AG. Increased accumulation of the glyoxidation product N-epsilon-(carboxymethyl)-lysine in human tissues in diabetes and aging. *J Clin Invest* 1997;**99**:457-68.
96. Ziegler D. Thioctic acid for patients with symptomatic diabetic polyneuropathy: a critical review. *Treat Endocrinol* 2004;**3**:173-89.
97. Ellman GL. Tissue sulphydryl groups. *Arch Biochem Biophys* 1959; **275**:175-84.
98. Tietze F. Enzymic method for the quantitative determination of nanogram amounts of total and oxidised glutathione. *Anal Biochem* 1969;**42**:64-70.
99. Reed DJ, Babson JR, Beatty PW, Brodie AE, Ellis WW, Potter DW. HPLC analysis of nanogram levels of glutathione, glutathione disulphide and related thiols and disulphides. *Anal Biochem* 1980;**106**:55-62.
100. Andersson A, Isaksson A, Bratstrom A, Hultberg B. Homocysteine and other thiols determined in plasma by HPLC and thiol specific post column derivatisation. *Clin Chem* 1993;**39**:1590-7.
101. Ueland PM, Mansoor MA, Guttormsen AB *et al.* Reduced, oxidised and protein-bound forms of homocysteine and other aminothiols in plasma comprise the redox thiol status – a possible element of the extracellular antioxidant defence system. *J Nutr* 1996;**126**:1281S-1284S.
102. Gracheva S, Livingstone C, Davis J. Development of a disposable potentiometric sensor for the near patient testing of plasma thiol concentrations. *Anal Chem* 2004;**76**:3833-6.
103. Gracheva S, Digga A, Livingstone C, Davis J. Selective potentiometric measurement of physiologically significant thiols. *Electroanalysis* 2005; **17**:205-09.